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(57) Abstract

Novel STRIFE1 and STRIFE2 polypeptides, proteins, and nucleic acid molecules are disclosed. In addition to isolated, full-length STRIFE1 and STRIFE2 proteins, the invention further provides isolated STRIFE1 and STRIFE2 fusion proteins, antigenic peptides and anti-STRIFE1 or STRIFE2 antibodies. The invention also provides STRIFE1 and STRIFE2 nucleic acid molecules, recombinant expression vectors containing nucleic acid molecules of the invention, host cells into which the expression vectors have been introduced and non-human transgenic animals in which a STRIFE1 or STRIFE2 gene has been introduced or disrupted. Diagnostic, screening and therapeutic methods utilizing compositions of the invention are also provided.

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NOVEL MOLECULES OF THE TNF RECEPTOR SUPERFAMILY AND USES THEREFOR

Background of the Invention

The tumor necrosis factor receptor (TNFR) superfamily of proteins encompasses over a dozen members, most of which are type I transmembrane proteins, related by the presence of conserved cysteine-rich repeats (CRRs) in their N-terminal cysteine-rich domains (CRDs). Members of the TNFR superfamily include TNFR1 (p55), TNFR2 (p75), TNFR3 (TNF-RP), Fas (also known as CD95 and Apo1), OX-40, 41-BB, CD40, CD30, CD27, OPG, and p75 NGFR. (Smith *et al.* (1993) *Cell* 76:959-962; Armitage, R.J. (1994) *Curr. Opin. Immunol.* 6:407-413; Gruss *et al.* (1995) *Blood* 85, 3378-3404; Baker *et al.* (1996) *Oncogene* 12:1-9; and Simonet *et al.* (1997) *Cell* 89:309-319.) A TNFR superfamily member is typically a membrane-bound, trimeric or multimeric complex which is stabilized via intracysteine disulfide bonds that are formed between the cysteine-rich domains of individual subunit members (Banner et al. (1993) *Cell* 73:431-445). The proteins themselves do not have intrinsic catalytic activity, rather they function via association with other proteins to transduce cellular signals.

A functional TNFR superfamily protein can also exist in a soluble form. Soluble versions of the superfamily bind cognate ligands and influence bioavailability. For instance, the osteoprotegerin protein family exists as a soluble protein. (Simonet *et al.* (1997) *Cell* 89:309-319.) Many soluble forms of the TNFR have been identified. Certain soluble TNFRs are elevated in disease states such as lupus and rheumatoid arthritis. (Gabay *et al.* (1997) *J. Rheumatol.* 24(2):303-308.) The soluble superfamily members lack the transmembrane domain characteristic of the majority of superfamily members due to either proteolytic cleavage or, at least in one instance, to alternative splicing (Gruss *et al.* (1995) *Blood* 85, 3378-3404.)

Summary of the Invention

The present invention is based, at least in part, on the discovery of novel molecules of the TNF receptor superfamily, referred to herein as "STRIFE" nucleic acid and protein molecules. Two splice forms of the "STRIFE" nucleic acid molecule have been identified and are referred to herein as the "STRIFE1" and "STRIFE2" nucleic acid and protein molecules. The STRIFE1 and STRIFE2 molecules of the present invention are useful as modulating agents in regulating a variety of cellular processes. Accordingly, in one aspect, this invention provides isolated nucleic acid molecules encoding STRIFE1 and STRIFE2 proteins or biologically active portions thereof, as

well as nucleic acid fragments suitable as primers or hybridization probes for the detection of STRIFE1 and STRIFE2-encoding nucleic acids.

In one embodiment, a STRIFE1 nucleic acid molecule is at least about 60%, 65%, 70%, 71%, 75%, 80%, 85%, 90%, 95%, 98% or more homologous to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, or a complement thereof. In yet another embodiment, a STRIFE2 nucleic acid molecule is at least about 60%, 65%, 70%, 71%, 75%, 80%, 85%, 90%, 95%, 98% or more homologous to the nucleotide sequence shown in SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:8, or a complement thereof. In a preferred embodiment, an isolated STRIFE1 nucleic acid molecule has the nucleotide sequence shown SEQ ID NO:3, or a complement thereof. In another embodiment, a STRIFE1 nucleic acid molecule further comprises nucleotides 1-106 of SEQ ID NO:1. In yet another preferred embodiment, a STRIFE1 nucleic acid molecule further comprises nucleotides 751-981 of SEQ ID NO:1. In another preferred embodiment, an isolated STRIFE1 nucleic acid molecule has the nucleotide sequence shown in SEQ ID NO:1.

In another preferred embodiment, an isolated STRIFE2 nucleic acid molecule has the nucleotide sequence shown SEQ ID NO:7, or a complement thereof. In another embodiment, a STRIFE2 nucleic acid molecule further comprises nucleotides 1-109 of SEQ ID NO:5. In yet another preferred embodiment, a STRIFE2 nucleic acid molecule further comprises nucleotides 562-655 of SEQ ID NO:5. In another preferred embodiment, an isolated STRIFE2 nucleic acid molecule has the nucleotide sequence shown in SEQ ID NO:5.

In another embodiment, a STRIFE1 or a STRIFE2 nucleic acid molecule include a nucleotide sequence encoding a protein having an amino acid sequence sufficiently homologous to the amino acid sequence of SEQ ID NO:2, or SEQ ID NO:6, respectively. In another preferred embodiment, a STRIFE1 or a STRIFE2 nucleic acid molecule include a nucleotide sequence encoding a protein having an amino acid sequence 60%, 65%, 70%, 75%, 80%, 85%, 86%, 90%, 95%, 98% or more homologous to the amino acid sequence of SEQ ID NO:2, or SEQ ID NO:6, respectively.

In another embodiment, an isolated nucleic acid molecule of the present invention encodes a STRIFE1 protein which includes a cysteine-rich domain, optionally a signal sequence, and is membrane bound. In another embodiment, an isolated nucleic acid molecule of the present invention encodes a STRIFE1 protein which includes a signal sequence and a cysteine-rich domain, wherein the cysteine-rich domain comprises at least one module, and is membrane bound. In yet another embodiment, a STRIFE1 nucleic acid molecule encodes a STRIFE1 protein and is a naturally occurring nucleotide sequence.

In another embodiment, an isolated nucleic acid molecule of the present invention encodes a STRIFE2 protein which includes a cysteine-rich domain, optionally a signal sequence, and is secreted. In another embodiment, an isolated nucleic acid molecule of the present invention encodes a STRIFE2 protein which includes a signal sequence and a cysteine-rich domain, wherein the cysteine-rich domain comprises at least one module, and is secreted. In yet another embodiment, a STRIFE2 nucleic acid molecule encodes a STRIFE2 protein and is a naturally occurring nucleotide sequence.

Another embodiment of the invention features STRIFE1 or STRIFE2 nucleic acid molecules which specifically detect STRIFE1 or STRIFE2 nucleic acid molecules, respectively, relative to nucleic acid molecules encoding non-STRIFE1 or non-STRIFE2 proteins. For example, in one embodiment, a STRIFE1 or STRIFE2 nucleic acid molecule hybridizes under stringent conditions to a nucleic acid molecule comprising the nucleotides 107-751, 1-16, 413-602, or 711-981 of the nucleotide sequence shown in SEQ ID NO:1, or to nucleotides 110-562, 1-16, 416-489, or 519-655 of nucleotide sequence shown in SEQ ID NO:5, respectively. In another embodiment, the STRIFE1 or STRIFE2 nucleic acid molecule is at least 450 nucleotides in length and hybridizes under stringent conditions to a nucleic acid molecule comprising the nucleotide sequence shown in SEQ ID NO:1, or SEQ ID NO:5, respectively, or a complement thereof.

Another embodiment of the invention provides an isolated nucleic acid molecule which is antisense to the coding strand of a STRIFE1 or a STRIFE2 nucleic acid molecule.

Another aspect of the invention provides a vector comprising a STRIFE1 or a STRIFE2 nucleic acid molecule. In certain embodiments, the vector is a recombinant expression vector. In another embodiment, the invention provides a host cell containing a vector of the invention. The invention also provides a method for producing a STRIFE1 or a STRIFE2 protein by culturing in a suitable medium, a host cell of the invention containing a recombinant expression vector such that a STRIFE1 or a STRIFE2 protein, respectively, is produced.

Another aspect of this invention features isolated or recombinant STRIFE1 or STRIFE2 proteins and polypeptides. In one embodiment, an isolated STRIFE1 protein has a cysteine-rich domain, optionally a signal sequence, and is membrane bound. In another embodiment, an isolated STRIFE2 protein has a cysteine-rich domain, optionally a signal sequence, and is secreted. In yet another embodiment, an isolated STRIFE1 or STRIFE2 protein has an amino acid sequence sufficiently homologous to the amino acid sequence of SEQ ID NO:2, or SEQ ID NO:6, respectively. In a preferred embodiment, a STRIFE1 protein has an amino acid sequence at least about 60%, 65%,

70%, 75%, 80%, 85%, 86%, 90%, 95%, 98% or more homologous to the amino acid sequence of SEQ ID NO:2. In another preferred embodiment, a STRIFE2 protein has an amino acid sequence at least about 60%, 65%, 70%, 75%, 80%, 85%, 86%, 90%, 95%, 98% or more homologous to the amino acid sequence of SEQ ID NO:6. In another embodiment, a STRIFE1 or a STRIFE2 protein has the amino acid sequence of SEQ ID NO:2, or SEQ ID NO:6, respectively.

Another embodiment of the invention features an isolated STRIFE1 protein which is encoded by a nucleic acid molecule having a nucleotide sequence at least about 60% homologous to a nucleotide sequence of SEQ ID NO:1, or a complement thereof. Another embodiment of the invention features an isolated STRIFE2 protein which is encoded by a nucleic acid molecule having a nucleotide sequence at least about 60% homologous to a nucleotide sequence of SEQ ID NO:5, or a complement thereof. This invention further features an isolated STRIFE1 or STRIFE2 protein which is encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, or SEQ ID NO:5, respectively, or a complement thereof.

The STRIFE1 and STRIFE2 proteins of the present invention, or biologically active portions thereof, can be operatively linked to a non-STRIFE1 and a non-STRIFE2 polypeptide to form STRIFE1 and STRIFE2 fusion proteins. The invention further features antibodies that specifically bind STRIFE1 and STRIFE2 proteins, such as monoclonal or polyclonal antibodies. In addition, the STRIFE1 and STRIFE2 proteins or biologically active portions thereof can be incorporated into pharmaceutical compositions, which optionally include pharmaceutically acceptable carriers.

In another aspect, the present invention provides a method for detecting STRIFE1 and STRIFE2 expression in a biological sample by contacting the biological sample with an agent capable of detecting a STRIFE1 and a STRIFE2 nucleic acid molecule, protein or polypeptide such that the presence of a STRIFE1 and a STRIFE2 nucleic acid molecule, protein or polypeptide is detected in the biological sample.

In another aspect, the present invention provides a method for detecting the presence of STRIFE1 and STRIFE2 activity in a biological sample by contacting the biological sample with an agent capable of detecting an indicator of STRIFE1 and STRIFE2 activity such that the presence of STRIFE1 and STRIFE2 activity is detected in the biological sample.

In another aspect, the invention provides a method for modulating STRIFE1 and STRIFE2 activity comprising contacting the cell with an agent that modulates STRIFE1 and/or STRIFE2 activity such that STRIFE1 and/or STRIFE2 activity in the cell is modulated. In one embodiment, the agent inhibits STRIFE1 and/or STRIFE2 activity.

In another embodiment, the agent stimulates STRIFE1 and/or STRIFE2 activity. In one embodiment, the agent is an antibody that specifically binds to a STRIFE1 and/or a STRIFE2 protein. In another embodiment, the agent modulates expression of STRIFE1 and STRIFE2 by modulating transcription of a STRIFE1 and a STRIFE2 gene or translation of a STRIFE1 and a STRIFE2 mRNA. In yet another embodiment, the agent is a nucleic acid molecule having a nucleotide sequence that is antisense to the coding strand of a STRIFE1 and a STRIFE2 mRNA or a STRIFE gene.

In one embodiment, the methods of the present invention are used to treat a subject having a disorder characterized by aberrant STRIFE1 and/or STRIFE2 protein or nucleic acid expression or activity by administering an agent which is a STRIFE1 and/or STRIFE2 modulator to the subject. In one embodiment, the STRIFE1 and STRIFE2 modulator is a STRIFE1 and a STRIFE2 protein, respectively. In another embodiment the STRIFE1 or STRIFE2 modulator is a STRIFE1 or a STRIFE2 nucleic acid molecule, respectively. In yet another embodiment, the STRIFE1 and the STRIFE2 modulator is a peptide, peptidomimetic, or other small molecule. In a preferred embodiment, the disorder characterized by aberrant STRIFE1 and/or STRIFE2 protein or nucleic acid expression is a developmental, differentiative, or proliferative disorder.

The present invention also provides a diagnostic assay for identifying the presence or absence of a genetic alteration characterized by at least one of (i) aberrant modification or mutation of a gene encoding a STRIFE1 and/or a STRIFE2 protein; (ii) mis-regulation of said gene; and (iii) aberrant post-translational modification of a STRIFE1 and/or a STRIFE2 protein, wherein a wild-type form of said gene encodes an protein with a STRIFE1 and a STRIFE2 activity, respectively.

In another aspect the invention provides a method for identifying a compound that binds to or modulates the activity of a STRIFE1 or a STRIFE2 protein, by providing an indicator composition comprising a STRIFE1 and/or STRIFE2 protein having STRIFE1 and/or STRIFE2 activity, respectively, contacting the indicator composition with a test compound, and determining the effect of the test compound on STRIFE1 or STRIFE2 activity in the indicator composition to identify a compound that modulates the activity of a STRIFE1 or a STRIFE2 protein, respectively.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

Brief Description of the Drawings

Figure 1 depicts the cDNA sequence and predicted amino acid sequence of murine STRIFE1. The nucleotide sequence corresponds to nucleic acids 1 to 981 of SEQ ID NO:1. The amino acid sequence corresponds to amino acids 1 to 214 of SEQ ID NO:2.

Figure 2 depicts the cDNA sequence and predicted amino acid sequence of murine STRIFE2. The nucleotide sequence corresponds to nucleic acids 1 to 655 of SEQ ID NO:5. The amino acid sequence corresponds to amino acids 1 to 150 of SEQ ID NO:6.

Figure 3 depicts an alignment of the amino acid sequences of murine STRIFE1 (also refered to herein as "Tango127a" or "T127a"), STRIFE2 (also refered to herein as "Tango127b" or "T127b"), and murine OX40 (Accesssion Number P47741). Amino acid residues which are conserved between murine STRIFE1 and STRIFE2 family members are highlighted.

Figure 4 depicts the results from a FASTA search using the amino acid sequence of STRIFE1 as a query.

Figure 5 depicts the results froma FASTA search using the nucleotide sequence of STRIFE1 as a query.

Detailed Description of the Invention

The present invention is based, at least in part, on the discovery of novel TNF receptor family members, referred to herein as "STRIFE1" and "STRIFE2" nucleic acid and protein molecules. TNF receptors are typically membrane-bound, trimeric or multimeric complexes which are stabilized via intracysteine disulfide bonds that are formed between the cysteine-rich domains of individual subunit members (Banner et al. (1993) *Cell* 73:431-445). Functional TNF receptors can also exist in a soluble form. Soluble members of the superfamily bind cognate ligands and influence bioavailability. The soluble superfamily members lack the transmembrane domain characteristic of the majority of superfamily members due to either proteolytic cleavage or, at least in one instance, to alternative splicing (Gruss *et al.* (1995) *Blood* 85, 3378-3404).

TNF receptors are the sole mediators of Tumor Necrosis Factor (TNF) signaling. TNF is a cytokine that is capable of acting independently or in conjunction with other factors to affect various different body functions. *In vitro*, TNF has diverse biological effects, including killing of transformed cells, stimulation of granulocytes and fibroblasts, damage to endothelial cells, and anti-parasitic effects. *In vivo*, TNF plays a key role as an endogenous mediator of inflammatory, immune, and host defense functions. In addition, TNF plays a role in various neoplastic disease states.

The STRIFE1 and STRIFE2 molecules of the present invention having homology to the TNF receptors may also be TNF receptors involved in TNF signaling. Thus, the STRIFE1 and STRIFE2 molecules of the present invention may play a role in mediating inflammatory, immune, and host defense functions. In addition, the STRIFE1 and STRIFE2 molecules of the present invention may play a role in various neoplastic disease states. Thus, the STRIFE1 and STRIFE2 molecules may be useful as targets for developing novel diagnostic and therapeutic agents to treat TNF-associated disorders and TNF receptor-associated disorders.

As used herein, the terms "TNF-associated disorder" and "TNF receptor-associated disorder" include any disorder, disease, or condition which is associated with an abnormal or undesired TNF or TNF receptor function or an abnormal or undesired TNF or TNF receptor level, e.g., plasma, tissue, or cellular levels or concentration. Examples of TNF-associated and TNF receptor-associated disorders include, but are not limited to, sepsis syndrome, including cachexia; circulatory collapse and shock resulting from acute or chronic bacterial infection; acute and chronic parasitic or infectious processes, including bacterial, viral and fungal infections; acute and chronic immune and autoimmune pathologies, such as systemic lupus erythematosus and rheumatoid arthritis; alcohol-induced hepatitis; chronic inflammatory pathologies such as sarcoidosis and Crohn's pathology; vascular inflammatory pathologies such as disseminated intravascular coagulation; graft-versus-host pathology; Rawasaki's pathology; malignant pathologies involving TNF-secreting tumors; cerebral malaria; and multiple sclerosis.

The term "family" when referring to the protein and nucleic acid molecules of the invention is intended to mean two or more proteins or nucleic acid molecules having a common structural domain and having sufficient amino acid or nucleotide sequence homology as defined herein. Such family members can be naturally occurring and can be from either the same or different species. For example, a family can contain a first protein of human origin, as well as other, distinct proteins of human origin or alternatively, can contain homologues of non-human origin. Members of a family may also have common functional characteristics.

In one embodiment, a STRIFE1 and a STRIFE2 family member is identified based on the presence of at least one "cysteine-rich domain" in the protein or corresponding nucleic acid molecule. As used herein, the term "cysteine-rich domain" refers to a protein domain of about 110-160 amino acid residues in length, preferably about 100-150 amino acid residues in length, more preferably about 90-140 amino acid residues in length, and even more preferably at least about 80-130 amino acid residues in length, of which at least about 10-30, preferably about 10-20, and more preferably about

12, 13, 14, or 15 amino acid residues are cysteine residues. In a preferred embodiment, a cysteine-rich domain is located in the N-terminal region of a STRIFE1 and STRIFE2 protein and includes about amino acid residues 34 through 114 of SEQ ID NO:2 and SEQ ID NO:6, respectively. Preferred cysteine rich domains contain at least about two, three, or four modules or motifs, wherein each module is a region of about 20-60 amino acid residues in length, preferably 30-50 amino acid residues in length, more preferably 40 amino acid residues in length and includes about 3-10 cysteines, preferably 5-7 cysteines, and more preferably 6 cysteines. In one embodiment, the module has the following motif:

C-Xaa1(4-14)-C-Xaa2(0-2)-C-Xaa3(2-4)-C-Xaa4(6-12)-C-Xaa5(6-10)-C(SEQ ID NO:17),

wherein "C" is the amino acid cysteine and "Xaa1-Xaa5" can be any amino acid residue. In a preferred embodiment, Xaa1 is between 4-6, 6-8, 8-10, 10-12, or 12-14 amino acid residues; Xaa4 is between 6-8, 8-10, or 10-12 amino acid residues; and Xaa5 is between 6-8 or 8-10 amino acid residues. In another preferred embodiment, Xaa1 is 4-6 amino acid residues, of which at least one is the amino acid phenylalanine, at least one is the amino acid tyrosine, and/or at least one is the amino acid histidine. In yet another preferred embodiment, Xaa5 is 6-10 amino acid residues, of which at least one is the amino acid aspartic acid, at least one is the amino acid asparagine, at least one is the amino acid glutamic acid, at least one is the amino acid glutamine, at least one is the amino acid serine, at least one is the amino acid lysine, and/or at least one is the amino acid proline. In another embodiment, the module has the following motif:

C-Xaa1(4,6)-FYH-Xaa2(5,10)-C-Xaa3(0,2)-C-Xaa4(2,3)-C-Xaa5(7,11)-C-Xaa6(4,6)-DNEQSKP-Xaa7(2)-C(SEQ ID NO:16).

For example, in one embodiment, a STRIFE1 protein contains a cysteine-rich domain including a first module containing about amino acids 34-72 of SEQ ID NO:2 (shown separately as SEQ ID NO:11) having 6 cysteine residues at positions indicated by the aforementioned motifs, and a second module containing about amino acids 75-114 of SEQ ID NO:2 (shown separately as SEQ ID NO:12) having 6 cysteine residues at positions indicated by the aforementioned motifs. In another embodiment, a STRIFE2 protein contains a cysteine rich domain including a first module containing about amino acids 34-72 of SEQ ID NO:6 (shown separately as SEQ ID NO:14) having 6 cysteine residues at positions indicated by the aforementioned motifs, and a second module containing about amino acids 75-114 of SEQ ID NO:6 (shown separately as SEQ ID NO:15) having 6 cysteine residues at positions indicated by the aforementioned motifs.

In another embodiment of the invention, a STRIFE1 and STRIFE2 protein has at least one cysteine-rich domain and a signal sequence. As used herein, a "signal sequence" refers to a peptide containing about 20 amino acids which occurs at the N-terminus of secretory and integral membrane proteins and which contains a large number of hydrophobic amino acid residues. For example, a signal sequence contains at least about 14-28 amino acid residues, preferably about 16-26 amino acid residues, more preferably about 18-24 amino acid residues, and more preferably about 20-22 amino acid residues, and has at least about 40-70%, preferably about 50-65%, and more preferably about 55-60% hydrophobic amino acid residues (e.g., Alanine, Valine, Leucine, Isoleucine, Phenylalanine, Tyrosine, Tryptophan, or Proline). Such a "signal sequence", also referred to in the art as a "signal peptide", serves to direct a protein containing such a sequence to a lipid bilayer. For example, in one embodiment, a STRIFE1 protein contains a signal sequence of about amino acids 1-29 of SEQ ID NO:2 (shown separately as SEQ ID NO:9). In another embodiment, a STRIFE2 protein contains a signal sequence of about amino acids 1-29 of SEQ ID NO:6 (shown separately as SEQ ID NO:13).

Accordingly, one embodiment of the invention features a STRIFE1 or a STRIFE2 protein having at least one cysteine-rich domain. Another embodiment features a STRIFE1 or a STRIFE2 protein having at least one cysteine-rich domain, wherein the cysteine-rich domain includes at least one module having the predicted motif of SEQ ID NO:16. Another embodiment features a STRIFE1 or a STRIFE2 protein having at least one cysteine-rich domain, wherein the cysteine-rich domain includes at least two modules. Another embodiment features a protein having 20, 30, 40, 50, 60, 70, 80, 90, 95, or 99% homology to a cysteine-rich domain of a STRIFE1 or a STRIFE2 protein of the invention.

Yet another embodiment of the invention features a STRIFE1 or a STRIFE2 protein having at least one cysteine-rich domain and a signal peptide. Another embodiment features a STRIFE1 or a STRIFE2 protein having at least one cysteine-rich domain and a signal peptide, wherein the cysteine-rich domain includes at least one module having the predicted motif of SEQ ID NO:16.

In yet another embodiment of the invention, a STRIFE1 protein has a transmembrane domain. As used herein, the term "transmembrane domain" refers to a structural amino acid motif which includes a hydrophobic helix that spans the plasma membrane. A transmembrane domain preferably includes a series of hydrophobic residues, such as leucine, valine, and tyrosine residues. For example, a STRIFE1 protein contains a transmembrane domain containing amino acids 169-193 of SEQ ID NO:2 (shown seperately as SEQ ID NO:10).

Preferred STRIFE1 or STRIFE2 molecules of the present invention have an amino acid sequence sufficiently homologous to the amino acid sequence of SEQ ID NO:2, or SEQ ID NO:6, respectively. As used herein, the term "sufficiently homologous" refers to a first amino acid or nucleotide sequence which contains a sufficient or minimum number of identical or equivalent (e.g., an amino acid residue which has a similar side chain) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences share common structural domains and/or a common functional activity. For example, amino acid or nucleotide sequences which share common structural domains have at least about 40% homology, preferably 50% homology, more preferably 60%-70% homology across the amino acid sequences of the domains and contain at least one, preferably two, more preferably three, and even more preferably four, five or six structural domains, are defined herein as sufficiently homologous. Furthermore, amino acid or nucleotide sequences which share at least 40%, preferably 50%, more preferably 60, 70, or 80% homology and share a common functional activity are defined herein as sufficiently homologous.

As used interchangeably herein, a "STRIFE1 and a STRIFE2 activity", "biological activity of STRIFE1 and STRIFE2" or "functional activity of STRIFE1 and STRIFE2", refers to an activity exerted by a STRIFE1 and a STRIFE2 protein, polypeptide or nucleic acid molecule on a STRIFE1 or a STRIFE2 responsive cell as determined *in vivo*, or *in vitro*, according to standard techniques. In one embodiment, a STRIFE1 and a STRIFE2 activity is a direct activity, such as an association with a STRIFE1 or a STRIFE2-target molecule. As used herein, a "target molecule" is a molecule with which a STRIFE1 and a STRIFE2 protein binds or interacts in nature, such that STRIFE1 or STRIFE2-mediated function is achieved. A STRIFE1 or a STRIFE2 target molecule can be a non-STRIFE1 and a non-STRIFE2 molecule or a STRIFE1 or STRIFE2 protein or polypeptide of the present invention. In an exemplary embodiment, a STRIFE2 target molecule is a membrane-bound protein (e.g., a "STRIFE2 receptor") or a modified form of such a protein which has been altered such that the protein is soluble (e.g., recombinantly produced such that the protein does not express a membrane-binding domain). In another embodiment, a STRIFE1 or a STRIFE2 target is a second soluble protein molecule (e.g., a "STRIFE1 or a STRIFE2 binding partner" or a "STRIFE1 and STRIFE2 substrate"). In such an exemplary embodiment, a STRIFE1 and a STRIFE2 binding partner can be a soluble non-STRIFE1 and non-STRIFE2 protein or a second STRIFE1 and a STRIFE2 protein molecule of the present invention. Alternatively, a STRIFE1 and a STRIFE2 activity is an indirect activity, such as a cellular signaling activity mediated by interaction of the STRIFE1 and

the STRIFE2 protein with a second protein (e.g., a STRIFE1 ligand or a STRIFE2 receptor).

In a preferred embodiment, a STRIFE1 activity is at least one or more of the following activities: (i) interaction of a STRIFE1 protein on the cell surface with a second non-STRIFE1 protein molecule on the surface of the same cell; (ii) interaction of a STRIFE1 protein on the cell surface with a second non-STRIFE1 protein molecule on the surface of a different cell; (iii) complex formation between a membrane-bound STRIFE1 protein and a cytokine, e.g., TNF; (iv) interaction of a STRIFE1 protein with an intracellular protein including SH2 domain-containing proteins or cytoskeletal proteins; (v) formation of a homogeneous multimeric signaling complex with STRIFE1-like proteins; and (vi) formation of a heterogeneous multimeric signaling complex with other TNFR superfamily proteins.

In another preferred embodiment, a STRIFE2 activity is at least one or more of the following activities: (i) interaction of a STRIFE2 protein with a membrane-bound STRIFE2 receptor; (ii) interaction of a STRIFE2 protein with a soluble form of a STRIFE2 receptor; (iii) interaction of a STRIFE2 protein with an intracellular protein via a membrane-bound STRIFE2 receptor; (iv) complex formation between a soluble STRIFE2 protein and a second soluble STRIFE2 binding partner; (v) complex formation between a soluble STRIFE2 protein and a second soluble STRIFE2 binding partner, wherein the STRIFE2 binding partner is a non-STRIFE2 protein molecule; and (vi) complex formation between a soluble STRIFE2 protein and a second soluble STRIFE2 binding partner, wherein the STRIFE2 binding partner is a second STRIFE2 protein molecule.

In yet another preferred embodiment, a STRIFE1 or a STRIFE2 activity is at least one or more of the following activities: (i) modulation of cellular signal transduction, either *in vitro or in vivo*; (ii) regulation of gene transcription in a cell involved in development or differentiation, either *in vitro or in vivo*; (iii) modulation of cellular signal transduction; (iv) regulation of cellular proliferation; (v) regulation of cellular differentiation; and (vi) regulation of cell survival.

Accordingly, another embodiment of the invention features isolated STRIFE1 and STRIFE2 proteins and polypeptides having a STRIFE1 and/or STRIFE2 activity, respectively. Preferred STRIFE1 and STRIFE2 proteins have at least one cysteine-rich domain and a STRIFE1 and/or a STRIFE2 activity. In another preferred embodiment, the STRIFE1 and STRIFE2 protein has at least one cysteine-rich domain, wherein the cysteine-rich domain comprises at least one module, and a STRIFE1 and STRIFE2 activity, respectively. In another preferred embodiment, the STRIFE1 and STRIFE2 protein has at least one cysteine-rich domain, wherein the cysteine-rich domain

comprises at least two modules, and a STRIFE1 and STRIFE2 activity, respectively. In yet another preferred embodiment, a STRIFE1 and a STRIFE2 protein further comprises a signal sequence. In still another preferred embodiment, a STRIFE1 and a STRIFE2 protein has a cysteine-rich domain, a STRIFE1 and a STRIFE2 activity, and an amino acid sequence sufficiently homologous to an amino acid sequence of SEQ ID NO:2, or SEQ ID NO:6, respectively.

The murine STRIFE1 cDNA, which is approximately 981 nucleotides in length, encodes a protein which is approximately 214 amino acid residues in length. The murine STRIFE1 protein contains an N-terminal signal sequence and a cysteine-rich domain comprising two modules. A STRIFE1 cysteine-rich domain can be found at least, for example, from about amino acids 34-114 of SEQ ID NO:2. The STRIFE1 cysteine-rich domain comprises a first module from about amino acids 34-72 of SEQ ID NO:2 (shown separately as SEQ ID NO:11) and a second module from about amino acids 75-114 of SEQ ID NO:2 (shown separately as SEQ ID NO:12). The murine STRIFE1 protein is a membrane bound protein which contains a transmembrane domain at about amino acids 169-193 of SEQ ID NO:2 (shown seperately as SEQ ID NO:10) and a signal sequence at about amino acids 1-29 of SEQ ID NO:2 (shown separately as SEQ ID NO:9). The prediction of such a signal peptide can be made, for example, utilizing the computer algorithm SIGNALP (Henrik, et al. (1997) *Protein Engineering* 10:1-6).

The murine STRIFE2 cDNA, which is approximately 655 nucleotides in length, encodes a protein which is approximately 150 amino acid residues in length. The murine STRIFE2 protein contains an N-terminal signal sequence and a cysteine-rich domain comprising two modules. A STRIFE2 cysteine-rich domain can be found at least, for example, from about amino acids 34-114 of SEQ ID NO:6. The STRIFE2 cysteine-rich domain comprises a first module from about amino acids 34-72 of SEQ ID NO:6 (shown separately as SEQ ID NO:14) and a second module from about amino acids 75-114 of SEQ ID NO:6 (shown separately as SEQ ID NO:15). The murine STRIFE2 protein is a secreted protein which further contains a signal sequence at about amino acids 1-29 of SEQ ID NO:6 (shown separately as SEQ ID NO:13).

Various aspects of the invention are described in further detail in the following subsections:

I. Isolated Nucleic Acid Molecules

One aspect of the invention pertains to isolated nucleic acid molecules which encode STRIFE1 and STRIFE2 proteins or biologically active portions thereof, as well as nucleic acid fragments sufficient for use as hybridization probes to identify STRIFE1

and STRIFE2-encoding nucleic acids (e.g., STRIFE1 and STRIFE2 mRNA) and fragments for use as PCR primers for the amplification or mutation of STRIFE1 and STRIFE2 nucleic acid molecules. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (e.g., cDNA or genomic DNA) and RNA molecules (e.g., mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

An "isolated" nucleic acid molecule is one which is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (i.e., sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated STRIFE1 and STRIFE2 nucleic acid molecule can contain less than about 5 kb, 4kb, 3kb, 2kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized.

A nucleic acid molecule of the present invention, e.g., a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:8 or a portion thereof, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or a portion of the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:8 as a hybridization probe, STRIFE1 and STRIFE2 nucleic acid molecules can be isolated using standard hybridization and cloning techniques (e.g., as described in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

Moreover, a nucleic acid molecule encompassing all or a portion of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:8 can be isolated by the polymerase chain reaction (PCR) using synthetic oligonucleotide primers designed based upon the sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:8.

A nucleic acid of the invention can be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to STRIFE1 and STRIFE2 nucleotide sequences can be prepared by standard synthetic techniques, e.g., using an automated DNA synthesizer.

In a preferred embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:1. The sequence of SEQ ID NO:1 corresponds to the murine STRIFE1 cDNA. This cDNA comprises sequences encoding the murine STRIFE1 protein (i.e., "the coding region", from nucleotides 107-751), as well as 5' untranslated sequences (nucleotides 1 to 106) and 3' untranslated sequences (nucleotides 752-981). Alternatively, the nucleic acid molecule can comprise only the coding region of SEQ ID NO:1 (e.g., nucleotides 107-751, corresponding to SEQ ID NO:3).

In another preferred embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:5. The sequence of SEQ ID NO:5 corresponds to the murine STRIFE2 cDNA. This cDNA comprises sequences encoding the murine STRIFE2 protein (i.e., "the coding region", from nucleotides 110-562), as well as 5' untranslated sequences (nucleotides 1-109) and 3' untranslated sequences (nucleotides 563-655). Alternatively, the nucleic acid molecule can comprise only the coding region of SEQ ID NO:5 (e.g., nucleotides 110-562, corresponding to SEQ ID NO:7).

In another preferred embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule which is a complement of the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID , or a portion of either of these nucleotide sequences. A nucleic acid molecule which is complementary to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:8 is one which is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:8 such that it can hybridize to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:8, thereby forming a stable duplex.

In still another preferred embodiment, an isolated nucleic acid molecule of the present invention comprises a nucleotide sequence which is at least about 60%, 65%, 70%, 71%, 75%, 80%, 85%, 90%, 95%, 98% or more homologous to the nucleotide

sequences show in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:8, or a portion of either of these nucleotide sequences larger than 450 bp.

Moreover, the nucleic acid molecule of the invention can comprise only a portion of the nucleic acid sequence of SEQ ID NO:1, or SEQ ID NO:5, for example a fragment which can be used as a probe or primer or a fragment encoding a biologically active portion of a STRIFE1 or a STRIFE2 protein. The nucleotide sequence determined from the cloning of the murine STRIFE1 and STRIFE2 genes allows for the generation of probes and primers designed for use in identifying and/or cloning STRIFE homologues in other cell types, e.g., from other tissues, as well as STRIFE homologues from other mammals including humans. The probe/primer typically comprises a substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12, preferably about 25, more preferably about 40, 50 or 75 consecutive nucleotides of a sense sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:8, of an antisense sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:8, or of a naturally occurring mutant of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:8. In an exemplary embodiment, a nucleic acid molecule of the present invention comprises a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising nucleotides 1-16, 413-602, or 711-981 of SEQ ID NO:1 or to a nucleic acid molecule comprising nucleotides 1-16, 416-489, or 519-655 of SEQ ID NO:5.

Probes based on the murine STRIFE1 and STRIFE2 nucleotide sequence can be used to detect transcripts or genomic sequences encoding the same or homologous proteins. In preferred embodiments, the probe further comprises a label group attached thereto, e.g., the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a diagnostic test kit for identifying cells or tissue which misexpress a STRIFE1 or a STRIFE2 protein, such as by measuring a level of a STRIFE1 or a STRIFE2-encoding nucleic acid in a sample of cells from a subject e.g., detecting STRIFE1 or STRIFE2 mRNA levels or determining whether a genomic STRIFE1 or STRIFE2 gene has been mutated or deleted.

A nucleic acid fragment encoding a "biologically active portion of a STRIFE1 or a STRIFE2 protein" can be prepared by isolating a portion of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:8 which encodes a polypeptide having a STRIFE1 or a STRIFE2 biological activity (the biological activities of the STRIFE1 and STRIFE2 proteins include biological activities attributed

to the TNFR super-family of proteins), expressing the encoded portion of the STRIFE1 or the STRIFE2 protein (e.g., by recombinant expression *in vitro*) and assessing the activity of the encoded portion of the STRIFE1 or STRIFE2 protein.

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:8 due to degeneracy of the genetic code and thus encode the same STRIFE1 or STRIFE2 proteins as those encoded by the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:8, respectively. In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a protein having an amino acid sequence shown in SEQ ID NO:2 or SEQ ID NO:6.

In addition to the murine STRIFE1 and STRIFE2 nucleotide sequences shown in SEQ ID NO:1 and SEQ ID NO:5, respectively, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of the STRIFE1 and STRIFE2 proteins may exist within a population (e.g., the human population). Such genetic polymorphism in the STRIFE1 or STRIFE2 genes may exist among individuals within a population due to natural allelic variation. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules comprising an open reading frame encoding a STRIFE1 or STRIFE2 protein, preferably a mammalian STRIFE1 or STRIFE2 protein. Such natural allelic variations can typically result in 1-5% variance in the nucleotide sequence of a STRIFE1 or a STRIFE2 gene. Any and all such nucleotide variations and resulting amino acid polymorphisms in STRIFE1 or STRIFE2 genes that are the result of natural allelic variation and that do not alter the functional activity of a STRIFE1 or STRIFE2 protein are intended to be within the scope of the invention.

Moreover, nucleic acid molecules encoding STRIFE1 and STRIFE2 proteins from other species, and thus which have a nucleotide sequence which differs from the murine sequence of SEQ ID NO:1 and SEQ ID NO:5 are intended to be within the scope of the invention.

Nucleic acid molecules corresponding to natural allelic variants and homologues of the STRIFE1 or STRIFE2 cDNAs of the invention can be isolated based on their homology to the murine STRIFE1 or STRIFE2 nucleic acids disclosed herein using the murine cDNA, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions.

Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 15 nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID

NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:8. In another embodiment, the nucleic acid is at least 30, 50, 100, 250 or 500 nucleotides in length. As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% homologous to each other typically remain hybridized to each other. Preferably, the conditions are such that sequences at least about 70%, more preferably at least about 80%, even more preferably at least about 85% or 90% homologous to each other typically remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. A preferred, non-limiting example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2 X SSC, 0.1% SDS at 50-65°C. Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of SEQ ID NO:1 corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (e.g., encodes a natural protein).

In addition to naturally-occurring allelic variants of the STRIFE1 or STRIFE2 sequences that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequences of SEQ ID NO:1, or SEQ ID NO:5, thereby leading to changes in the amino acid sequence of the encoded STRIFE1 or STRIFE2 proteins, without altering the functional ability of the STRIFE1 or STRIFE2 proteins. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in the sequence of SEQ ID NO:1, or SEQ ID NO:5. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of STRIFE1 or STRIFE2 (e.g., the sequence of SEQ ID NO:2 or SEQ ID NO:6) without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. For example, amino acid residues that are conserved among the STRIFE1 or STRIFE2 proteins of the present invention, are predicted to be particularly unamenable to alteration. Furthermore, amino acid residues that are conserved between STRIFE1 or STRIFE2 protein and other proteins having cysteine-rich domains are not likely to be amenable to alteration.

Accordingly, another aspect of the invention pertains to nucleic acid molecules encoding STRIFE1 or STRIFE2 proteins that contain changes in amino acid residues that are not essential for activity. Such STRIFE1 or STRIFE2 proteins differ in amino acid sequence from SEQ ID NO:2 or SEQ ID NO:6 yet retain biological activity. In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence

encoding a protein, wherein the protein comprises an amino acid sequence at least about 60% homologous to the amino acid sequence of SEQ ID NO:2, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, or SEQ ID NO:15. Preferably, the protein encoded by the nucleic acid molecule is at least about 60%, 65%, 70%, 75%, 80%, 85%, 86%, 90%, 95%, 98% or more homologous to SEQ ID NO:2, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, or SEQ ID NO:15.

An isolated nucleic acid molecule encoding a STRIFE1 or STRIFE2 protein homologous to the protein of SEQ ID NO:2 or SEQ ID NO:6, respectively, can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, or SEQ ID NO:7, such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations can be introduced into SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, or SEQ ID NO:7 by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in a STRIFE1 or STRIFE2 protein is preferably replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a STRIFE1 or STRIFE2 coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for STRIFE1 or STRIFE2 biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:8, the encoded protein can be expressed recombinantly and the activity of the protein can be determined.

In a preferred embodiment, a mutant STRIFE1 or STRIFE2 protein can be assayed for the ability to (i) modulate cellular signal transduction, either *in vitro or in vivo*; (ii) regulate gene transcription in a cell involved in development or differentiation, either *in vitro or in vivo*; (iii) modulate cellular signal transduction; (iv) regulate cellular

proliferation; (v) regulate cellular differentiation; (vi) regulate cell survival; and (vii) modulate a cell involved in the immune response.

In addition to the nucleic acid molecules encoding STRIFE1 or STRIFE2 proteins described above, another aspect of the invention pertains to isolated nucleic acid molecules which are antisense thereto. An "antisense" nucleic acid comprises a nucleotide sequence which is complementary to a "sense" nucleic acid encoding a protein, e.g., complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid. The antisense nucleic acid can be complementary to an entire STRIFE1 or STRIFE2 coding strand, or to only a portion thereof. In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding STRIFE1 or STRIFE2. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues (e.g., the coding region of murine STRIFE1 corresponds to SEQ ID NO:3 and the coding region of murine STRIFE2 corresponds to SEQ ID NO:7). In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding STRIFE1 or STRIFE2. The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (i.e., also referred to as 5' and 3' untranslated regions).

Given the coding strand sequences encoding STRIFE1 or STRIFE2 disclosed herein (e.g., SEQ ID NO:3 or SEQ ID NO:7), antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of STRIFE1 or STRIFE2 mRNA, but more preferably is an oligonucleotide which is antisense to only a portion of the coding or noncoding region of STRIFE1 or STRIFE2 mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of STRIFE1 or STRIFE2 mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-

fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5- oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a STRIFE1 or STRIFE2 protein to thereby inhibit expression of the protein, e.g., by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention include direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, e.g., by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an α-anomeric nucleic acid molecule. An α-anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the

usual β-units, the strands run parallel to each other (Gaultier et al. (1987) *Nucleic Acids. Res.* 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue et al. (1987) *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue et al. (1987) *FEBS Lett.* 215:327-330).

In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (e.g., hammerhead ribozymes (described in Haselhoff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave STRIFE1 or STRIFE2 mRNA transcripts to thereby inhibit translation of STRIFE1 or STRIFE2 mRNA. A ribozyme having specificity for a STRIFE1 or STRIFE2-encoding nucleic acid can be designed based upon the nucleotide sequence of a STRIFE1 or STRIFE2 cDNA disclosed herein (i.e., SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:8). For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a STRIFE1 or STRIFE2-encoding mRNA. See, e.g., Cech et al. U.S. Patent No. 4,987,071; and Cech et al. U.S. Patent No. 5,116,742. Alternatively, STRIFE1 or STRIFE2 mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, e.g., Bartel, D. and Szostak, J.W. (1993) *Science* 261:1411-1418.

Alternatively, STRIFE1 or STRIFE2 gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the STRIFE1 or STRIFE2 (e.g., the STRIFE1 or STRIFE2 promoter and/or enhancers) to form triple helical structures that prevent transcription of the STRIFE1 or STRIFE2 gene in target cells. See generally, Helene, C. (1991) *Anticancer Drug Des.* 6(6):569-84; Helene, C. et al. (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher, L.J. (1992) *Bioassays* 14(12):807-15.

In yet another embodiment, the STRIFE1 or STRIFE2 nucleic acid molecules of the present invention can be modified at the base moiety, sugar moiety or phosphate backbone to improve, e.g., the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acid molecules can be modified to generate peptide nucleic acids (see Hyrup B. *et al.* (1996) *Bioorganic & Medicinal Chemistry* 4 (1): 5-23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, e.g., DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for

specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup B. *et al.* (1996) *supra*; Perry-O'Keefe *et al.* PNAS 93: 14670-675.

PNAs of STRIFE1 or STRIFE2 nucleic acid molecules can be used for therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, for example, inducing transcription or translation arrest or inhibiting replication. PNAs of STRIFE1 or STRIFE2 nucleic acid molecules can also be used in the analysis of single base pair mutations in a gene, (e.g., by PNA-directed PCR clamping); as 'artificial restriction enzymes' when used in combination with other enzymes, (e.g., S1 nucleases (Hyrup B. (1996) *supra*)); or as probes or primers for DNA sequencing or hybridization (Hyrup B. *et al.* (1996) *supra*; Perry-O'Keefe *supra*).

In another embodiment, PNAs of STRIFE1 or STRIFE2 can be modified, (e.g., to enhance their stability or cellular uptake), by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras of STRIFE1 or STRIFE2 nucleic acid molecules can be generated which may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, (e.g., RNAse H and DNA polymerases), to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (Hyrup B. (1996) *supra*). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup B. (1996) *supra* and Finn P.J. *et al.* (1996) *Nucleic Acids Res.* 24 (17): 3357-63. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry and modified nucleoside analogs, e.g., 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used as a between the PNA and the 5' end of DNA (Mag, M. *et al.* (1989) *Nucleic Acid Res.* 17: 5973-88). PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn P.J. *et al.* (1996) *supra*). Alternatively, chimeric moleclues can be synthesized with a 5' DNA segment and a 3' PNA segment (Peterser, K.H. *et al.* (1975) *Bioorganic Med. Chem. Lett.* 5: 1119-11124).

In other embodiments, the oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, e.g., Letsinger *et al.* (1989) *Proc. Natl. Acad. Sci. US.* 86:6553-6556; Lemaitre *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:648-652;

PCT Publication No. W088/09810, published December 15, 1988) or the blood-brain barrier (see, e.g., PCT Publication No. W089/10134, published April 25, 1988). In addition, oligonucleotides can be modified with hybridization-triggered cleavage agents (See, e.g., Krol *et al.* (1988) *BioTechniques* 6:958-976) or intercalating agents. (See, e.g., Zon (1988) *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, (e.g., a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent).

II. Isolated STRIFE1 and STRIFE2 Proteins and Anti-STRIFE1 and -STRIFE2 Antibodies

One aspect of the invention pertains to isolated STRIFE1 and STRIFE2 proteins, and biologically active portions thereof, as well as polypeptide fragments suitable for use as immunogens to raise anti-STRIFE1 and STRIFE2 antibodies. In one embodiment, native STRIFE1 or STRIFE2 proteins can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, STRIFE1 or STRIFE2 proteins are produced by recombinant DNA techniques. Alternative to recombinant expression, a STRIFE1 or STRIFE2 protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

An "isolated" or "purified" protein or biologically active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the STRIFE1 or STRIFE2 protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of STRIFE1 or STRIFE2 protein in which the protein is separated from cellular components of the cells from which it is isolated or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations of STRIFE1 or STRIFE2 protein having less than about 30% (by dry weight) of non-STRIFE1 or STRIFE2 protein (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-STRIFE1 or non-STRIFE2 protein, still more preferably less than about 10% of non-STRIFE1 or non-STRIFE2 protein, and most preferably less than about 5% non-STRIFE1 or non-STRIFE2 protein. When the STRIFE1 or STRIFE2 protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, i.e., culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation.

The language "substantially free of chemical precursors or other chemicals" includes preparations of STRIFE1 or STRIFE2 protein in which the protein is separated from chemical precursors or other chemicals which are involved in the synthesis of the protein. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of STRIFE1 or STRIFE2 protein having less than about 30% (by dry weight) of chemical precursors or non-STRIFE1 or non-STRIFE2 chemicals, more preferably less than about 20% chemical precursors or non-STRIFE1 or non-STRIFE2 chemicals, still more preferably less than about 10% chemical precursors or non-STRIFE1 or non-STRIFE2 chemicals, and most preferably less than about 5% chemical precursors or non-STRIFE1 or non-STRIFE2 chemicals.

Biologically active portions of a STRIFE1 or STRIFE2 protein include peptides comprising amino acid sequences sufficiently homologous to or derived from the amino acid sequence of the STRIFE1 or STRIFE2 protein, e.g., the amino acid sequence shown in SEQ ID NO:2 or SEQ ID NO:6, which include less amino acids than the full length STRIFE1 or STRIFE2 proteins, and exhibit at least one activity of a STRIFE1 or STRIFE2 protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the STRIFE1 or STRIFE2 protein. A biologically active portion of a STRIFE1 or STRIFE2 protein can be a polypeptide which is, for example, 10, 25, 50, 100 or more amino acids in length.

In one embodiment, a biologically active portion of a STRIFE1 or STRIFE2 protein comprises at least a cysteine-rich domain. In another embodiment, a biologically active portion of a STRIFE1 or STRIFE2 protein comprises at least a cysteine-rich domain, wherein the cysteine-domain includes at least one module. In yet another embodiment, a biologically active portion of a STRIFE1 or STRIFE2 protein comprises at least a signal sequence. In yet a further embodiment, a biologically active portion of a STRIFE1 or STRIFE2 protein comprises at least a cysteine-rich domain and a signal sequence.

In an alternative embodiment, a biologically active portion of a STRIFE1 or STRIFE2 protein comprises a STRIFE1 or STRIFE2 amino acid sequence lacking a signal sequence. In another alternative embodiment, a biologically active portion of a STRIFE1 or STRIFE2 protein comprises a STRIFE1 or STRIFE2 amino acid sequence lacking a cysteine-rich domain.

It is to be understood that a preferred biologically active portion of a STRIFE1 or STRIFE2 protein of the present invention may contain at least one of the above-identified structural domains. Another preferred biologically active portion of a STRIFE1 or STRIFE2 protein may contain at least two of the above-identified structural domains. Another more preferred biologically active portion of a STRIFE1 or

STRIFE2 protein may contain at least three or more of the above-identified structural domains.

Moreover, other biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native STRIFE1 or STRIFE2 protein.

In a preferred embodiment, the STRIFE1 or STRIFE2 protein has an amino acid sequence shown in SEQ ID NO:2 or SEQ ID NO:6, respectively. In other embodiments, the STRIFE1 or STRIFE2 protein is substantially homologous to SEQ ID NO:2 or SEQ ID NO:6, and retains the functional activity of the protein of SEQ ID NO:2 or SEQ ID NO:6, respectively, yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail in subsection I above. Accordingly, in another embodiment, the STRIFE1 or STRIFE2 protein is a protein which comprises an amino acid sequence at least about 60% homologous to the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:6 and preferably retains a functional activity of the STRIFE1 or STRIFE2 protein of SEQ ID NO:2 or SEQ ID NO:6, respectively. Preferably, the protein is at least about 70% homologous to SEQ ID NO:2 or SEQ ID NO:6, more preferably at least about 80% homologous to SEQ ID NO:2 or SEQ ID NO:6, even more preferably at least about 90% homologous to SEQ ID NO:2 or SEQ ID NO:6, and most preferably at least about 95% or more homologous to SEQ ID NO:2 or SEQ ID NO:6.

To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, or 90% of the length of the reference sequence (e.g., when aligning a second sequence to the STRIFE1 and STRIFE2 amino acid sequence of SEQ ID NO:2 or SEQ ID NO:6 having 177 amino acid residues, at least 80, preferably at least 100, more preferably at least 120, even more preferably at least 140, and even more preferably at least 150, 160 or 170 amino acid residues are aligned). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by

the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at http://www.gcg.com), using either a Blossom 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at http://www.gcg.com), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. In another embodiment, the percent identity between two amino acid or nucleotide sequences is determined using the algorithm of E. Meyers and W. Miller (CABIOS, 4:11-17 (1989)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

The nucleic acid and protein sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, et al. (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to STRIFE1 or STRIFE2 nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to STRIFE1 or STRIFE2 protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See http://www.ncbi.nlm.nih.gov.

The invention also provides STRIFE1 or STRIFE2 chimeric or fusion proteins. As used herein, a STRIFE1 or STRIFE2 "chimeric protein" or "fusion protein" comprises a STRIFE1 or STRIFE2 polypeptide operatively linked to a non-STRIFE1 or non-STRIFE2 polypeptide. A "STRIFE1 or STRIFE2 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to STRIFE1 or STRIFE2, whereas a "non-STRIFE1 or non-STRIFE2 polypeptide" refers to a polypeptide having

an amino acid sequence corresponding to a protein which is not substantially homologous to the STRIFE1 or STRIFE2 protein, e.g., a protein which is different from the STRIFE1 or STRIFE2 protein and which is derived from the same or a different organism. Within a STRIFE1 or STRIFE2 fusion protein the STRIFE1 or STRIFE2 polypeptide can correspond to all or a portion of a STRIFE1 or STRIFE2 protein. In a preferred embodiment, a STRIFE1 or STRIFE2 fusion protein comprises at least one biologically active portion of a STRIFE1 or STRIFE2 protein. In another preferred embodiment, a STRIFE1 or STRIFE2 fusion protein comprises at least two biologically active portions of a STRIFE1 or STRIFE2 protein. In another preferred embodiment, a STRIFE1 or STRIFE2 fusion protein comprises at least three biologically active portions of a STRIFE1 or STRIFE2 protein. Within the fusion protein, the term "operatively linked" is intended to indicate that the STRIFE1 or STRIFE2 polypeptide and the non-STRIFE1 or non-STRIFE2 polypeptide are fused in-frame to each other. The non-STRIFE1 or non-STRIFE2 polypeptide can be fused to the N-terminus or C-terminus of the STRIFE1 or STRIFE2 polypeptide.

For example, in one embodiment, the fusion protein is a GST-STRIFE1 or STRIFE2 fusion protein in which the STRIFE1 or STRIFE2 sequences are fused to the C-terminus of the GST sequences. Such fusion proteins can facilitate the purification of recombinant STRIFE1 or STRIFE2. In another embodiment, the fusion protein is a STRIFE1 or STRIFE2 protein containing a heterologous signal sequence at its N-terminus. For example, the native STRIFE1 or STRIFE2 signal sequence (i.e, about amino acids 1-29 of SEQ ID NO:2 or SEQ ID NO:6) can be removed and replaced with a signal sequence from another protein. In certain host cells (e.g., mammalian host cells), expression and/or secretion of STRIFE1 or STRIFE2 can be increased through use of a heterologous signal sequence.

In yet another embodiment, the fusion protein is a STRIFE1 or STRIFE2-immunoglobulin fusion protein in which the STRIFE1 or STRIFE2 sequences comprising primarily the STRIFE1 or STRIFE2 cysteine-rich domains are fused to sequences derived from a member of the immunoglobulin protein family. Soluble derivatives have also been made of cell surface glycoproteins in the immunoglobulin gene superfamily consisting of an extracellular domain of the cell surface glycoprotein fused to an immunoglobulin constant (Fc) region (see e.g., Capon, D.J. *et al.* (1989) *Nature* 337:525-531 and Capon U.S. Patents 5,116,964 and 5,428,130 [CD4-IgG1 constructs]; Linsley, P.S. *et al.* (1991) *J. Exp. Med.* 173:721-730 [a CD28-IgG1 construct and a B7-1-IgG1 construct]; and Linsley, P.S. *et al.* (1991) *J. Exp. Med.* 174:561-569 and U.S. Patent 5,434,131[a CTLA4-IgG1]). Such fusion proteins have proven useful for modulating receptor-ligand interactions. Soluble derivatives of cell

surface proteins of the tumor necrosis factor receptor (TNFR) superfamily proteins have been made consisting of an extracellular domain of the cell surface receptor fused to an immunoglobulin constant (Fc) region (see for example Moreland *et al.* (1997) N. Engl. J. Med. 337(3):141-147; van der Poll *et al.* (1997) Blood 89(10):3727-3734; and Ammann *et al.* (1997) J. Clin. Invest. 99(7):1699-1703).

The STRIFE1 or STRIFE2-immunoglobulin fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject to inhibit an interaction between a STRIFE1 ligand and a STRIFE1 receptor on the surface of a cell, or between a STRIFE2 receptor and the STRIFE2 ligand, to thereby suppress STRIFE1 or STRIFE2-mediated signal transduction *in vivo*. The STRIFE1 or STRIFE2-immunoglobulin fusion proteins can be used to affect the bioavailability of a STRIFE1 or STRIFE2 cognate receptor. Inhibition of the STRIFE1 or STRIFE2 ligand/STRIFE1 or STRIFE2 interaction may be useful therapeutically for the treatment of TNF-associated disorders, e.g., inflammatory, immune, or neoplastic disorders. Moreover, the STRIFE1 or STRIFE2-immunoglobulin fusion proteins of the invention can be used as immunogens to produce anti-STRIFE1 or STRIFE2 antibodies in a subject, to purify STRIFE1 or STRIFE2 ligands and in screening assays to identify molecules which inhibit the interaction of STRIFE1 or STRIFE2 with a STRIFE1 or STRIFE2 ligand.

Preferably, a STRIFE1 or STRIFE2 chimeric or fusion protein of the invention is produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, for example by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, *Current Protocols in Molecular Biology*, eds. Ausubel et al. John Wiley & Sons: 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (e.g., a GST polypeptide). A STRIFE1 or STRIFE2-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the STRIFE1 or STRIFE2 protein.

The present invention also pertains to variants of the STRIFE1 or STRIFE2 proteins which function as either STRIFE1 or STRIFE2 agonists (mimetics) or as STRIFE1 or STRIFE2 antagonists. Variants of the STRIFE1 or STRIFE2 proteins can be generated by mutagenesis, e.g., discrete point mutation or truncation of a STRIFE1 or STRIFE2 protein. An agonist of the STRIFE1 or STRIFE2 proteins can retain substantially the same, or a subset, of the biological activities of the naturally occurring form of a STRIFE1 or STRIFE2 protein. An antagonist of a STRIFE1 or STRIFE2 protein can inhibit one or more of the activities of the naturally occurring form of the STRIFE1 or STRIFE2 protein by, for example, competitively binding to a downstream or upstream member of a cellular signaling cascade which includes the STRIFE1 or STRIFE2 protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. In one embodiment, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the STRIFE1 or STRIFE2 protein.

In one embodiment, variants of a STRIFE1 or STRIFE2 protein which function as either STRIFE1 or STRIFE2 agonists (mimetics) or as STRIFE1 or STRIFE2 antagonists can be identified by screening combinatorial libraries of mutants, e.g., truncation mutants, of a STRIFE1 or STRIFE2 protein for STRIFE1 or STRIFE2 protein agonist or antagonist activity. In one embodiment, a variegated library of STRIFE1 or STRIFE2 variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of STRIFE1 or STRIFE2 variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential STRIFE1 or STRIFE2 sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (e.g., for phage display) containing the set of STRIFE1 or STRIFE2 sequences therein. There are a variety of methods which can be used to produce libraries of potential STRIFE1 or STRIFE2 variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential STRIFE1 or STRIFE2 sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (see, e.g., Narang, S.A. (1983) *Tetrahedron* 39:3; Itakura et al. (1984) *Annu. Rev. Biochem.* 53:323; Itakura et al. (1984) *Science* 198:1056; Ike et al. (1983) *Nucleic Acid Res.* 11:477.

In addition, libraries of fragments of a STRIFE1 or STRIFE2 protein coding sequence can be used to generate a variegated population of STRIFE1 or STRIFE2 fragments for screening and subsequent selection of variants of a STRIFE1 or STRIFE2 protein. In one embodiment, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of a STRIFE1 or STRIFE2 coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA which can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes N-terminal, C-terminal and internal fragments of various sizes of the STRIFE1 or STRIFE2 protein.

Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of STRIFE1 or STRIFE2 proteins. The most widely used techniques, which are amenable to high through-put analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recrusive ensemble mutagenesis (REM), a new technique which enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify STRIFE1 or STRIFE2 variants (Arkin and Yourvan (1992) *PNAS 89*:7811-7815; Delgrave et al. (1993) *Protein Engineering* 6(3):327-331).

In one embodiment, cell based assays can be exploited to analyze a variegated STRIFE1 or STRIFE2 library. For example, a library of expression vectors can be transfected into a cell line which ordinarily responds to a particular ligand, e.g., a cytokine, in a STRIFE1 or STRIFE2-dependent manner. The transfected cells are then contacted with the ligand and the effect of expression of the mutant on signaling by the ligand can be detected, e.g., by measuring NF-κB activity or cell survival. Plasmid DNA can then be recovered from the cells which score for inhibition, or alternatively, potentiation of cytokine induction, and the individual clones further characterized.

An isolated STRIFE1 or STRIFE2 protein, or a portion or fragment thereof, can be used as an immunogen to generate antibodies that bind STRIFE1 or STRIFE2 using standard techniques for polyclonal and monoclonal antibody preparation. A full-length

STRIFE1 or STRIFE2 protein can be used or, alternatively, the invention provides antigenic peptide fragments of STRIFE1 or STRIFE2 for use as immunogens. The antigenic peptide of STRIFE1 or STRIFE2 comprises at least 8 amino acid residues of the amino acid sequence shown in SEQ ID NO:2 or SEQ ID NO:6 and encompasses an epitope of STRIFE1 or STRIFE2 such that an antibody raised against the peptide forms a specific immune complex with STRIFE1 or STRIFE2. Preferably, the antigenic peptide comprises at least 10 amino acid residues, more preferably at least 15 amino acid residues, even more preferably at least 20 amino acid residues, and most preferably at least 30 amino acid residues.

Preferred epitopes encompassed by the antigenic peptide are regions of STRIFE1 or STRIFE2 that are located on the surface of the protein, e.g., hydrophilic regions.

A STRIFE1 or STRIFE2 immunogen typically is used to prepare antibodies by immunizing a suitable subject, (e.g., rabbit, goat, mouse or other mammal) with the immunogen. An appropriate immunogenic preparation can contain, for example, recombinantly expressed STRIFE1 or STRIFE2 protein or a chemically synthesized STRIFE1 or STRIFE2 polypeptide. The preparation can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or similar immunostimulatory agent. Immunization of a suitable subject with an immunogenic STRIFE1 or STRIFE2 preparation induces a polyclonal anti-STRIFE1 or STRIFE2 antibody response.

Accordingly, another aspect of the invention pertains to anti-STRIFE1 or STRIFE2 antibodies. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site which specifically binds (immunoreacts with) an antigen, such as STRIFE1 or STRIFE2. Examples of immunologically active portions of immunoglobulin molecules include F(ab) and $F(ab')_2$ fragments which can be generated by treating the antibody with an enzyme such as pepsin. The invention provides polyclonal and monoclonal antibodies that bind STRIFE1 or STRIFE2. The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of STRIFE1 or STRIFE2. A monoclonal antibody composition thus typically displays a single binding affinity for a particular STRIFE1 or STRIFE2 protein with which it immunoreacts.

Polyclonal anti-STRIFE1 or STRIFE2 antibodies can be prepared as described above by immunizing a suitable subject with a STRIFE1 or STRIFE2 immunogen. The anti-STRIFE1 or STRIFE2 antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized STRIFE1 or STRIFE2. If desired, the antibody molecules

directed against STRIFE1 or STRIFE2 can be isolated from the mammal (e.g., from the blood) and further purified by well known techniques, such as protein A chromatography to obtain the IgG fraction. At an appropriate time after immunization, e.g., when the anti-STRIFE1 or STRIFE2 antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) *Nature* 256:495-497) (see also, Brown et al. (1981) *J. Immunol.* 127:539-46; Brown et al. (1980) *J. Biol. Chem* .255:4980-83; Yeh et al. (1976) *PNAS* 76:2927-31; and Yeh et al. (1982) *Int. J. Cancer* 29:269-75), the more recent human B cell hybridoma technique (Kozbor et al. (1983) *Immunol Today* 4:72), the EBV-hybridoma technique (Cole et al. (1985), *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology for producing monoclonal antibody hybridomas is well known (see generally R. H. Kenneth, in *Monoclonal Antibodies: A New Dimension In Biological Analyses*, Plenum Publishing Corp., New York, New York (1980); E. A. Lerner (1981) *Yale J. Biol. Med.*, 54:387-402; M. L. Gefter et al. (1977) *Somatic Cell Genet.* 3:231-36). Briefly, an immortal cell line (typically a myeloma) is fused to lymphocytes (typically splenocytes) from a mammal immunized with a STRIFE1 or STRIFE2 immunogen as described above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a monoclonal antibody that binds STRIFE1 or STRIFE2.

Any of the many well known protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating an anti-STRIFE1 or STRIFE2 monoclonal antibody (see, e.g., G. Galfre et al. (1977) *Nature* 266:55052; Gefter et al. *Somatic Cell Genet.*, cited *supra*; Lerner, *Yale J. Biol. Med.*, cited *supra*; Kenneth, *Monoclonal Antibodies*, cited *supra*). Moreover, the ordinarily skilled worker will appreciate that there are many variations of such methods which also would be useful. Typically, the immortal cell line (e.g., a myeloma cell line) is derived from the same mammalian species as the lymphocytes. For example, murine hybridomas can be made by fusing lymphocytes from a mouse immunized with an immunogenic preparation of the present invention with an immortalized mouse cell line. Preferred immortal cell lines are mouse myeloma cell lines that are sensitive to culture medium containing hypoxanthine, aminopterin and thymidine ("HAT medium"). Any of a number of myeloma cell lines can be used as a fusion partner according to standard techniques, e.g., the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 myeloma lines. These myeloma lines are available from ATCC. Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using polyethylene glycol ("PEG").

Hybridoma cells resulting from the fusion are then selected using HAT medium, which kills unfused and unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridoma cells producing a monoclonal antibody of the invention are detected by screening the hybridoma culture supernatants for antibodies that bind STRIFE1 or STRIFE2, e.g., using a standard ELISA assay.

Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal anti-STRIFE1 or STRIFE2 antibody can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (e.g., an antibody phage display library) with STRIFE1 or STRIFE2 to thereby isolate immunoglobulin library members that bind STRIFE1 or STRIFE2. Kits for generating and screening phage display libraries are commercially available (e.g., the Pharmacia *Recombinant Phage Antibody System*, Catalog No. 27-9400-01; and the Stratagene *SurfZAP™ Phage Display Kit*, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, Ladner et al. U.S. Patent No. 5,223,409; Kang et al. PCT International Publication No. WO 92/18619; Dower et al. PCT International Publication No. WO 91/17271; Winter et al. PCT International Publication WO 92/20791; Markland et al. PCT International Publication No. WO 92/15679; Breitling et al. PCT International Publication WO 93/01288; McCafferty et al. PCT International Publication No. WO 92/01047; Garrard et al. PCT International Publication No. WO 92/09690; Ladner et al. PCT International Publication No. WO 90/02809; Fuchs et al. (1991) *Bio/Technology* 9:1370-1372; Hay et al. (1992) *Hum. Antibod. Hybridomas* 3:81-85; Huse et al. (1989) *Science* 246:1275-1281; Griffiths et al. (1993) *EMBO J* 12:725-734; Hawkins et al. (1992) *J. Mol. Biol.* 226:889-896; Clarkson et al. (1991) *Nature* 352:624-628; Gram et al. (1992) *PNAS* 89:3576-3580; Garrad et al. (1991) *Bio/Technology* 9:1373-1377; Hoogenboom et al. (1991) *Nuc. Acid Res.* 19:4133-4137; Barbas et al. (1991) *PNAS* 88:7978-7982; and McCafferty et al. *Nature* (1990) 348:552-554.

Additionally, recombinant anti-STRIFE1 or STRIFE2 antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in Robinson et al. International Application No. PCT/US86/02269; Akira, et al. European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison et al. European Patent Application 173,494; Neuberger et al. PCT International Publication No. WO 86/01533; Cabilly et al. U.S. Patent No.

4,816,567; Cabilly et al. European Patent Application 125,023; Better et al. (1988) *Science* 240:1041-1043; Liu et al. (1987) *PNAS* 84:3439-3443; Liu et al. (1987) *J. Immunol.* 139:3521-3526; Sun et al. (1987) *PNAS* 84:214-218; Nishimura et al. (1987) *Canc. Res.* 47:999-1005; Wood et al. (1985) *Nature* 314:446-449; and Shaw et al. (1988) *J. Natl. Cancer Inst.* 80:1553-1559); Morrison, S. L. (1985) *Science* 229:1202-1207; Oi et al. (1986) *BioTechniques* 4:214; Winter U.S. Patent 5,225,539; Jones et al. (1986) *Nature* 321:552-525; Verhoeyan et al. (1988) *Science* 239:1534; and Beidler et al. (1988) *J. Immunol.* 141:4053-4060.

An anti-STRIFE1 or STRIFE2 antibody (e.g., monoclonal antibody) can be used to isolate STRIFE1 or STRIFE2 by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-STRIFE1 or STRIFE2 antibody can facilitate the purification of natural STRIFE1 or STRIFE2 from cells and of recombinantly produced STRIFE1 or STRIFE2 expressed in host cells. Moreover, an anti-STRIFE1 or STRIFE2 antibody can be used to detect STRIFE1 or STRIFE2 protein (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the STRIFE1 or STRIFE2 protein. Anti-STRIFE1 or STRIFE2 antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, e.g., to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (i.e., physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S or ^{3}H.

III. Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding STRIFE1 or STRIFE2 (or a portion thereof). As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional

DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (e.g., in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to includes promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel; *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in many types of host cell and those which direct expression of the nucleotide sequence only in certain host cells (e.g., tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (e.g., STRIFE1 or STRIFE2 proteins, mutant forms of STRIFE1 or STRIFE2, fusion proteins, etc.).

The recombinant expression vectors of the invention can be designed for expression of STRIFE1 or STRIFE2 in prokaryotic or eukaryotic cells. For example, STRIFE1 or STRIFE2 can be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promotors directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D.B. and Johnson, K.S. (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Purified fusion proteins can be utilized in STRIFE1 or STRIFE2 activity assays, in STRIFE1 or STRIFE2 ligand binding (e.g., direct assays or competitive assays described in detail below), to generate antibodies specific for STRIFE1 or STRIFE2 proteins, as examples. In a preferred embodiment, a STRIFE1 or STRIFE2 fusion expressed in a retroviral expression vector of the present invention can be utilized to infect bone marrow cells which are subsequently transplanted into irradiated recipients. The pathology of the subject recipient is then examined after sufficient time has passed (e.g six (6) weeks).

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amann et al., (1988) *Gene* 69:301-315) and pET 11d (Studier et al., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 60-89). Target gene expression from the pTrc vector relies on host RNA polymerase transcription from a hybrid trp-lac fusion promoter. Target gene expression from the pET 11d vector relies on transcription from a T7 gn10-lac fusion

promoter mediated by a coexpressed viral RNA polymerase (T7 gn1). This viral polymerase is supplied by host strains BL21(DE3) or HMS174(DE3) from a resident λ prophage harboring a T7 gn1 gene under the transcriptional control of the lacUV 5 promoter.

One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein (Gottesman, S., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 119-128). Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (Wada et al., (1992) *Nucleic Acids Res.* 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the STRIFE1 or STRIFE2 expression vector is a yeast expression vector. Examples of vectors for expression in yeast *S. cerivisae* include pYepSec1 (Baldari, et al., (1987) *Embo J.* 6:229-234), pMFa (Kurjan and Herskowitz, (1982) *Cell* 30:933-943), pJRY88 (Schultz et al., (1987) *Gene* 54:113-123), pYES2 (Invitrogen Corporation, San Diego, CA), and picZ (InVitrogen Corp, San Diego, CA).

Alternatively, STRIFE1 or STRIFE2 can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (e.g., Sf 9 cells) include the pAc series (Smith et al. (1983) *Mol. Cell Biol.* 3:2156-2165) and the pVL series (Lucklow and Summers (1989) *Virology* 170:31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, B. (1987) *Nature* 329:840) and pMT2PC (Kaufman et al. (1987) *EMBO J.* 6:187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see chapters 16 and 17 of Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid). Tissue-

specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert et al. (1987) *Genes Dev.* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv. Immunol.* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) *EMBO J.* 8:729-733) and immunoglobulins (Banerji et al. (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters (e.g., the neurofilament promoter; Byrne and Ruddle (1989) *PNAS* 86:5473-5477), pancreas-specific promoters (Edlund et al. (1985) *Science* 230:912-916), and mammary gland-specific promoters (e.g., milk whey promoter; U.S. Patent No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, for example the murine hox promoters (Kessel and Gruss (1990) *Science* 249:374-379) and the α-fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev.* 3:537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner which allows for expression (by transcription of the DNA molecule) of an RNA molecule which is antisense to STRIFE1 or STRIFE2 mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen which direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes see Weintraub, H. et al., Antisense RNA as a molecular tool for genetic analysis, *Reviews - Trends in Genetics*, Vol. 1(1) 1986.

Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, STRIFE1 or STRIFE2 protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (e.g., DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, et al. (*Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (e.g., resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred selectable markers include those which confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding STRIFE1 or STRIFE2 or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (e.g., cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (i.e., express) STRIFE1 or STRIFE2 protein. Accordingly, the invention further provides methods for producing STRIFE1 or STRIFE2 protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding STRIFE1 or STRIFE2 has been introduced) in a suitable medium such that STRIFE1 or STRIFE2 protein is produced. In another embodiment, the method further comprises isolating STRIFE1 or STRIFE2 from the medium or the host cell.

The host cells of the invention can also be used to produce nonhuman transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which STRIFE1 or STRIFE2-coding sequences have been introduced. Such host cells can then be used to create non-human transgenic animals in which exogenous STRIFE1 or STRIFE2 sequences have been introduced into

their genome or homologous recombinant animals in which endogenous STRIFE1 or STRIFE2 sequences have been altered. Such animals are useful for studying the function and/or activity of STRIFE1 or STRIFE2 and for identifying and/or evaluating modulators of STRIFE1 or STRIFE2 activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, etc. A transgene is exogenous DNA which is integrated into the genome of a cell from which a transgenic animal develops and which remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous STRIFE1 or STRIFE2 gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, e.g., an embryonic cell of the animal, prior to development of the animal.

A transgenic animal of the invention can be created by introducing STRIFE1 or STRIFE2-encoding nucleic acid into the male pronuclei of a fertilized oocyte, e.g., by microinjection, retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal. The murine STRIFE1 or STRIFE2 cDNA sequence of SEQ ID NO:1 or SEQ ID NO:4 can be introduced as a transgene into the genome of a non-human animal. Alternatively, a nonmurine homologue of a murine STRIFE1 or STRIFE2 gene, such as a human STRIFE1 or STRIFE2 gene, can be isolated based on hybridization to the murine STRIFE1 or STRIFE2 cDNA (described further in subsection I above) and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to the STRIFE1 or STRIFE2 transgene to direct expression of STRIFE1 or STRIFE2 protein to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009, both by Leder et al., U.S. Patent No. 4,873,191 by Wagner et al. and in Hogan, B., *Manipulating the Mouse Embryo*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986). Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of the STRIFE1 or STRIFE2 transgene in its genome and/or expression of STRIFE1 or STRIFE2 mRNA in tissues or cells of the animals. A transgenic founder

animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding STRIFE1 or STRIFE2 can further be bred to other transgenic animals carrying other transgenes.

To create a homologous recombinant animal, a vector is prepared which contains at least a portion of a STRIFE1 or STRIFE2 gene into which a deletion, addition or substitution has been introduced to thereby alter, e.g., functionally disrupt, the STRIFE1 or STRIFE2 gene. The STRIFE1 or STRIFE2 gene can be a murine gene (e.g., the cDNA of SEQ ID NO:3 or SEQ ID NO:7), but can also be a non-murine homologue of a murine STRIFE1 or STRIFE2 gene. For example, a human STRIFE1 or STRIFE2 gene can be used to construct a homologous recombination vector suitable for altering an endogenous STRIFE1 or STRIFE2 gene in the mouse genome. In a preferred embodiment, the vector is designed such that, upon homologous recombination, the endogenous STRIFE1 or STRIFE2 gene is functionally disrupted (i.e., no longer encodes a functional protein; also referred to as a "knock out" vector). Alternatively, the vector can be designed such that, upon homologous recombination, the endogenous STRIFE1 or STRIFE2 gene is mutated or otherwise altered but still encodes functional protein (e.g., the upstream regulatory region can be altered to thereby alter the expression of the endogenous STRIFE1 or STRIFE2 protein). In the homologous recombination vector, the altered portion of the STRIFE1 or STRIFE2 gene is flanked at its 5' and 3' ends by additional nucleic acid of the STRIFE1 or STRIFE2 gene to allow for homologous recombination to occur between the exogenous STRIFE1 or STRIFE2 gene carried by the vector and an endogenous STRIFE1 or STRIFE2 gene in an embryonic stem cell. The additional flanking STRIFE1 or STRIFE2 nucleic acid is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5' and 3' ends) are included in the vector (see e.g., Thomas, K.R. and Capecchi, M. R. (1987) *Cell* 51:503 for a description of homologous recombination vectors). The vector is introduced into an embryonic stem cell line (e.g., by electroporation) and cells in which the introduced STRIFE1 or STRIFE2 gene has homologously recombined with the endogenous STRIFE1 or STRIFE2 gene are selected (see e.g., Li, E. et al. (1992) *Cell* 69:915). The selected cells are then injected into a blastocyst of an animal (e.g., a mouse) to form aggregation chimeras (see e.g., Bradley, A. in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E.J. Robertson, ed. (IRL, Oxford, 1987) pp. 113-152). A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously recombined DNA by germline transmission of the

transgene. Methods for constructing homologous recombination vectors and homologous recombinant animals are described further in Bradley, A. (1991) *Current Opinion in Biotechnology* 2:823-829 and in PCT International Publication Nos.: WO 90/11354 by Le Mouellec et al.; WO 91/01140 by Smithies et al.; WO 92/0968 by Zijlstra et al.; and WO 93/04169 by Berns et al.

In another embodiment, transgenic non-humans animals can be produced which contain selected systems which allow for regulated expression of the transgene. One example of such a system is the *cre/loxP* recombinase system of bacteriophage P1. For a description of the *cre/loxP* recombinase system, see, e.g., Lakso et al. (1992) *PNAS* 89:6232-6236. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae* (O'Gorman et al. (1991) *Science* 251:1351-1355. If a *cre/loxP* recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the *Cre* recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, e.g., by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut, I. et al. (1997) *Nature* 385:810-813 and PCT International Publication Nos. WO 97/07668 and WO 97/07669. In brief, a cell, e.g., a somatic cell, from the transgenic animal can be isolated and induced to exit the growth cycle and enter G_0 phase. The quiescent cell can then be fused, e.g., through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The recontructed oocyte is then cultured such that it develops to morula or blastocyte and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell, e.g., the somatic cell, is isolated.

IV. Pharmaceutical Compositions

The STRIFE1 or STRIFE2 nucleic acid molecules, STRIFE1 or STRIFE2 proteins, and anti-STRIFE1 or STRIFE2 antibodies (also referred to herein as "active compounds") of the invention can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known

in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (e.g., a STRIFE1 or STRIFE2 protein or anti-STRIFE1 or STRIFE2 antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds which exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the

method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (i.e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by stereotactic injection (see e.g., Chen et al. (1994) *PNAS* 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, e.g., retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

V. Uses and Methods of the Invention

The nucleic acid molecules, proteins, protein homologues, and antibodies described herein can be used in one or more of the following methods: a) screening assays; b) detecting assays (e.g., chromosome mapping, tissue typing, and forensic biology); c) predictive medicine (e.g., diagnostic assays, prognostic assays, monitoring clinical trials, and pharmacogenetics); and d) methods of treatment (e.g., therapeutic and prophylactic methods as well as such methods in the context of pharmacogenomics). As described herein, a STRIFE1 protein of the invention has one or more of the following activities: (i) interaction of a STRIFE1 protein on the cell surface with a second non-STRIFE1 protein molecule on the surface of the same cell; (ii) interaction of a STRIFE1 protein on the cell surface with a second non-STRIFE1 protein molecule on the surface of a different cell; (iii) complex formation between a membrane-bound STRIFE1 protein and a cytokine, e.g., TNF; (iv) interaction of a STRIFE1 protein with an intracellular protein including SH2 domain-containing proteins or cytoskeletal proteins; (v) formation of a homogeneous multimeric signaling complex with like STRIFE1 proteins; and (vi) formation of a heterogeneous multimeric signaling complex with other TNFR superfamily proteins. As described herein, STRIFE2 protein of the invention has one or

more of the following activities: (i) interaction of a STRIFE2 protein with a membrane-bound STRIFE2 receptor; (ii) interaction of a STRIFE2 protein with a soluble form of a STRIFE2 receptor; (iii) interaction of a STRIFE2 protein with an intracellular protein via a membrane-bound STRIFE2 receptor; (iv) complex formation between a soluble STRIFE2 protein and a second soluble STRIFE2 binding partner; (v) complex formation between a soluble STRIFE2 protein and a second soluble STRIFE2 binding partner, wherein the STRIFE2 binding partner is a non-STRIFE2 protein molecule; and (vi) complex formation between a soluble STRIFE2 protein and a second soluble STRIFE2 binding partner, wherein the STRIFE2 binding partner is a second STRIFE2 protein molecule. The STRIFE1 and STRIFE2 proteins of the invention can can thus be used in, for example, (1) modulation of cellular signal transduction, either *in vitro or in vivo*; (2) regulation of gene transcription in a cell involved in development or differentiation, either *in vitro or in vivo*; (3) regulation of gene transcription in a cell involved in in development or differentiation, wherein at least one gene encodes a differentiation-specific protein; (4) regulation of gene transcription in a cell involved in in development or differentaition, wherein at least one gene encodes a second secreted protein; (5) regulation of gene transcription in a cell involved in development or differentiation, wherein at least one gene encodes a signal transduction molecule; and (6) regulation of cellular proliferation, either *in vitro or in vivo*. The isolated nucleic acid molecules of the invention can be used, for example, to express STRIFE1 or STRIFE2 protein (e.g., via a recombinant expression vector in a host cell in gene therapy applications), to detect STRIFE1 or STRIFE2 mRNA (e.g., in a biological sample) or a genetic alteration in a STRIFE1 or STRIFE2 gene, and to modulate STRIFE1 or STRIFE2 activity, as described further below. In addition, the STRIFE1 or STRIFE2 proteins can be used to screen drugs or compounds which modulate the STRIFE1 or STRIFE2 activity as well as to treat disorders characterized by insufficient or excessive production of STRIFE1 or STRIFE2 protein or production of STRIFE1 or STRIFE2 protein forms which have decreased or aberrant activity compared to STRIFE1 or STRIFE2 wild type protein (e.g., developmental disorders or proliferative diseases such as cancer). Moreover, the anti-STRIFE1 or STRIFE2 antibodies of the invention can be used to detect and isolate STRIFE1 or STRIFE2 proteins, regulate the bioavailability of STRIFE1 or STRIFE2 proteins, and modulate STRIFE1 or STRIFE2 activity.

A. Screening Assays:

The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, i.e., candidate or test compounds or agents (e.g., peptides, peptidomimetics, small molecules or other drugs) which bind to STRIFE1 or STRIFE2

proteins or have a stimulatory or inhibitory effect on, for example, STRIFE1 or STRIFE2 expression or STRIFE1 or STRIFE2 activity.

There are assays that can be used to identify candidate or test compounds or agents which have a stimulatory or inhibitory effect on, for example, STRIFE1 or STRIFE2 expression or STRIFE1 or STRIFE2 activity. For example, assays based on the effects of TNF on some cells can be used to evaluate the modulatory activity of test compounds on STRIFE1 or STRIFE2 expression or STRIFE1 or STRIFE2 activity. Known effects of TNF on fibroblast cells include effects on mitogenesis, IL-6 secretion and HLA class II antigen induction. Known effects of TNF on monocytes include effects on secretion of cytokines such as GM-CSF, IL-6, and IL-8. TNF is known to be cytotoxic to some cells, such as WEHI-164 murine fibrosarcoma cells (described in Espevik et al. (1986) *J. Immunol. Methods* 95:99-105). TNF is also known to have effects on cytokine secretion by endothelial cells, as well as effect induction of adhesion molecules such as ICAM-1, E-selectin, VCAM, and tissue factor production in endothelial cells. Thus, these cells and the detectable phenotypic changes resulting from the effect of TNF in the presence or absence of a test compound can be used to evaluate the modulatory activity of the test compound on STRIFE1 or STRIFE2 expression or STRIFE1 or STRIFE2 activity. Furthermore, TNF is known to modulate neutrophil responses. Comparisons can be made between TNF effects on neutrophils in the presence or absence of a test compound using cellular activation, priming, degranulation, and/or superoxide production as detectable endpoints for evaluation of STRIFE1 or STRIFE2 modulatory activity. These and other related assays are well known to those having ordinary skill in the art.

In one embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of a STRIFE1 or STRIFE2 protein or polypeptide or biologically active portion thereof. In another embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of a STRIFE1 receptor. The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K.S. (1997) *Anticancer Drug Des.* 12:145).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6909; Erb *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann *et al.* (1994). *J. Med. Chem.* 37:2678; Cho et al. (1993) *Science* 261:1303; Carrell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and in Gallop *et al.* (1994) *J. Med. Chem.* 37:1233.

Libraries of compounds may be presented in solution (e.g., Houghten (1992) *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria (Ladner USP 5,223,409), spores (Ladner USP '409), plasmids (Cull *et al.* (1992) *Proc Natl Acad Sci USA* 89:1865-1869) or on phage (Scott and Smith (1990) *Science* 249:386-390); (Devlin (1990) *Science* 249:404-406); (Cwirla *et al.* (1990) *Proc. Natl. Acad. Sci.* 87:6378-6382); (Felici (1991) *J. Mol. Biol.* 222:301-310); (Ladner *supra.*).

In one embodiment, an assay is a cell-based assay in which a cell which expresses a STRIFE1 or STRIFE2 receptor on the cell surface is contacted with a test compound and the ability of the test compound to bind to a STRIFE1 or STRIFE2 receptor is determined. The cell preferably expresses a human STRIFE1 or STRIFE2 receptor, e.g., the human receptor encoded by clone AX92_3 contained in ATCC Deposit Number 98101 (described in PCT application number WO 98/01554, published on January 15, 1998) or the human OAF065 receptor (described in PCT application number WO 98/38304, published on September 3, 1998). The cell, for example, can be of mammalian origin or a yeast cell. Determining the ability of the test compound to bind to a STRIFE1 or STRIFE2 receptor can be accomplished, for example, by coupling the test compound with a radioisotope or enzymatic label such that binding of the test compound to the STRIFE1 or STRIFE2 receptor can be determined by detecting the labeled compound in a complex. For example, test compounds can be labeled with ^{125}I, ^{35}S, ^{14}C, or ^{3}H, either directly or indirectly, and the radioisotope detected by direct counting of radioemmission or by scintillation counting. Alternatively, test compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

It is also within the scope of this invention to determine the ability of a test compound to interact with a STRIFE1 or STRIFE2 receptor without the labeling of any of the interactants. For example, a microphysiometer can be used to detect the interaction of a test compound with a STRIFE1 or STRIFE2 receptor without the labeling of either the test compound or the receptor. McConnell, H. M. *et al.* (1992) *Science* 257:1906-1912. As used herein, a "microphysiometer" (e.g., Cytosensor™) is

an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between ligand and receptor.

In a preferred embodiment, the assay comprises contacting a cell which expresses a STRIFE1 or STRIFE2 receptor on the cell surface with a STRIFE1 or STRIFE2 protein or biologically-active portion thereof, to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a STRIFE1 or STRIFE2 receptor, wherein determining the ability of the test compound to interact with a STRIFE1 or STRIFE2 receptor comprises determining the ability of the test compound to preferentially bind to the STRIFE1 or STRIFE2 receptor as compared to the ability of STRIFE1 or STRIFE2, or a biologically active portion thereof, to bind to the receptor.

In another embodiment, an assay is a cell-based assay comprising contacting a cell which expresses a STRIFE1 or STRIFE2 target molecule with a test compound and determining the ability of the test compound to modulate the activity of the STRIFE1 or STRIFE2 target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (e.g., intracellular Ca^{2+}, diacylglycerol, IP_3, etc.), detecting catalytic/enzymatic activity of the target an appropriate substrate, detecting the induction of a reporter gene (comprising a STRIFE1 or STRIFE2-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, e.g., luciferase), or detecting a cellular response, for example, development, differentiation or rate of proliferation.

In yet another embodiment, an assay of the present invention is a cell-free assay in which a STRIFE1 or STRIFE2 protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to bind to the STRIFE1 or STRIFE2 protein or biologically active portion thereof is determined. Binding of the test compound to the STRIFE1 or STRIFE2 protein can be determined either directly or indirectly as described above. In a preferred embodiment, the assay includes contacting the STRIFE1 or STRIFE2 protein or biologically active portion thereof with a known compound which binds STRIFE1 or STRIFE2 to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a STRIFE1 or STRIFE2 protein, wherein determining the ability of the test compound to interact with a STRIFE1 or STRIFE2 protein comprises determining the ability of the test compound to preferentially bind to STRIFE1 or STRIFE2 or biologically active portion thereof as compared to the known compound.

In another embodiment, the assay is a cell-free assay in which a STRIFE1 or STRIFE2 protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to modulate (e.g., stimulate or inhibit) the activity of the STRIFE1 or STRIFE2 protein or biologically active portion thereof is determined. Determining the ability of the test compound to modulate the activity of a STRIFE1 or STRIFE2 protein can be accomplished, for example, by determining the ability of the STRIFE1 or STRIFE2 protein to bind to a STRIFE1 or STRIFE2 target molecule by one of the methods described above for determining direct binding. Determining the ability of the STRIFE1 or STRIFE2 protein to bind to a STRIFE1 or STRIFE2 target molecule can also be accomplished using a technology such as real-time Biomolocular Interaction Analysis (BIA). Sjolander, S. and Urbaniczky, C. (1991) *Anal. Chem.* 63:2338-2345 and Szabo *et al.* (1995) *Curr. Opin. Struct. Biol.* 5:699-705. As used herein, "BIA" is a technology for studying biospecific interactions in real time, without labeling any of the interactants (e.g., BIAcore™). Changes in the optical phenomenon surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

In an alternative embodiment, determining the ability of the test compound to modulate the activity of a STRIFE1 or STRIFE2 protein can be accomplished by determining the ability of the STRIFE1 or STRIFE2 protein to further modulate the activity of a STRIFE1 or STRIFE2 target molecule. For example, the catalytic/enzymatic activity of the target molecule on an appropriate substrate can be determined as previously described.

In yet another embodiment, the cell-free assay involves contacting a STRIFE1 or STRIFE2 protein or biologically active portion thereof with a known compound which binds the STRIFE1 or STRIFE2 protein to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with the STRIFE1 or STRIFE2 protein, wherein determining the ability of the test compound to interact with the STRIFE1 or STRIFE2 protein comprises determining the ability of the STRIFE1 or STRIFE2 protein to preferentially bind to or modulate the activity of a STRIFE1 or STRIFE2 target molecule.

The cell-free assays of the present invention are amenable to use of both soluble and/or membrane-bound forms of isolated proteins (e.g., STRIFE1 or STRIFE2 proteins or biologically active portions thereof or STRIFE1 or STRIFE2 target molecules). In the case of cell-free assays in which a membrane-bound form an isolated protein is used (e.g., a STRIFE2 target molecule or receptor) it may be desirable to utilize a solubilizing agent such that the membrane-bound form of the isolated protein is maintained in solution. Examples of such solubilizing agents include non-ionic detergents such as n-

octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton® X-100, Triton® X-114, Thesit®, Isotridecypoly(ethylene glycol ether)$_n$, 3-[(3-cholamidopropyl)dimethylamminio]-1-propane sulfonate (CHAPS), 3-[(3-cholamidopropyl)dimethylamminio]-2-hydroxy-1-propane sulfonate (CHAPSO), or N-dodecyl=N,N-dimethyl-3-ammonio-1-propane sulfonate.

In more than one embodiment of the above assay methods of the present invention, it may be desirable to immobilize either STRIFE1 or STRIFE2 or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to a STRIFE1 or STRIFE2 protein, or interaction of a STRIFE1 or STRIFE2 protein with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtitre plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase/ STRIFE1 or STRIFE2 fusion proteins or glutathione-S-transferase/target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtitre plates, which are then combined with the test compound or the test compound and either the non-adsorbed target protein or STRIFE1 or STRIFE2 protein, and the mixture incubated under conditions conducive to complex formation (e.g., at physiological conditions for salt and pH). Following incubation, the beads or microtitre plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of STRIFE1 or STRIFE2 binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either a STRIFE1 or STRIFE2 protein or a STRIFE1 or STRIFE2 target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated STRIFE1 or STRIFE2 protein or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well known in the art (e.g., biotinylation kit, Pierce Chemicals, Rockford, IL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with STRIFE1 or STRIFE2 protein or target molecules but which do not interfere with binding of the STRIFE1 or STRIFE2 protein to its target molecule can be derivatized to the wells of the plate, and unbound target or STRIFE1 or

STRIFE2 protein trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the STRIFE1 or STRIFE2 protein or target molecule, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the STRIFE1 or STRIFE2 protein or target molecule.

In another embodiment, modulators of STRIFE1 or STRIFE2 expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of STRIFE1 or STRIFE2 mRNA or protein in the cell is determined. The level of expression of STRIFE1 or STRIFE2 mRNA or protein in the presence of the candidate compound is compared to the level of expression of STRIFE1 or STRIFE2 mRNA or protein in the absence of the candidate compound. The candidate compound can then be identified as a modulator of STRIFE1 or STRIFE2 expression based on this comparison. For example, when expression of STRIFE1 or STRIFE2 mRNA or protein is greater (statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of STRIFE1 or STRIFE2 mRNA or protein expression. Alternatively, when expression of STRIFE1 or STRIFE2 mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of STRIFE1 or STRIFE2 mRNA or protein expression. The level of STRIFE1 or STRIFE2 mRNA or protein expression in the cells can be determined by methods described herein for detecting STRIFE1 or STRIFE2 mRNA or protein.

In yet another aspect of the invention, the STRIFE1 or STRIFE2 proteins can be used as "bait proteins" in a two-hybrid assay or three-hybrid assay (see, e.g., U.S. Patent No. 5,283,317; Zervos *et al.* (1993) *Cell* 72:223-232; Madura *et al.* (1993) *J. Biol. Chem.* 268:12046-12054; Bartel et al. (1993) *Biotechniques* 14:920-924; Iwabuchi *et al.* (1993) *Oncogene* 8:1693-1696; and Brent WO94/10300), to identify other proteins, which bind to or interact with STRIFE1 or STRIFE2 ("STRIFE1- or STRIFE2-binding proteins" or "STRIFE1 or STRIFE2-bp") and modulate STRIFE1 or STRIFE2 activity. Such STRIFE1-binding proteins are also likely to be involved in the propagation of signals by the STRIFE1 proteins as, for example, downstream elements of a STRIFE1-mediated signaling pathway. Alternatively, such STRIFE2-binding proteins are likely to be cell-surface molecules associated with non-STRIFE2-expressing cells, wherein such STRIFE2-binding proteins are involved in signal transduction.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for a

STRIFE1 or STRIFE2 protein is fused to a gene encoding the DNA binding domain of a known transcription factor (e.g., GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, *in vivo,* forming an STRIFE1 or STRIFE2-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (e.g., LacZ) which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene which encodes the protein which interacts with the STRIFE1 or STRIFE2 protein.

This invention further pertains to novel agents identified by the above-described screening assays. Accordingly, it is within the scope of this invention to further use an agent identified as described herein in an appropriate animal model. For example, an agent identified as described herein (e.g., a STRIFE1 or STRIFE2 modulating agent, an antisense STRIFE1 or STRIFE2 nucleic acid molecule, a STRIFE1 or STRIFE2-specific antibody, or a STRIFE1 or STRIFE2-binding partner) can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can be used in an animal model to determine the mechanism of action of such an agent. Furthermore, this invention pertains to uses of novel agents identified by the above-described screening assays for treatments as described herein.

In the screening assays described herein, either the murine STRIFE1 or STRIFE2 receptors could be used, or preferably, a human STRIFE1 or STRIFE2 receptor, e.g., the human receptor encoded by clone AX92_3 contained in ATCC Deposit Number 98101 (described in PCT application number WO 98/01554, published on January 15, 1998) or the human OAF065 receptor (described in PCT application number WO 98/38304, published on September 3, 1998), may be used.

B. Detection Assays

Portions or fragments of the cDNA sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. For example, these sequences can be used to: (i) map their respective genes on a chromosome; and, thus, locate gene regions associated with genetic disease; (ii) identify an individual from a minute biological sample (tissue

typing); and (iii) aid in forensic identification of a biological sample. These applications are described in the subsections below.

1. Chromosome Mapping

Once the sequence (or a portion of the sequence) of a gene has been isolated, this sequence can be used to map the location of the gene on a chromosome. This process is called chromosome mapping. Accordingly, portions or fragments of the STRIFE1 or STRIFE2 nucleotide sequences, described herein, can be used to map the location of the STRIFE1 or STRIFE2 genes on a chromosome. The mapping of the STRIFE1 or STRIFE2 sequences to chromosomes is an important first step in correlating these sequences with genes associated with disease.

Briefly, STRIFE1 or STRIFE2 genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the STRIFE1 or STRIFE2 nucleotide sequences. Computer analysis of the STRIFE1 or STRIFE2 sequences can be used to predict primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers can then be used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the STRIFE1 or STRIFE2 sequences will yield an amplified fragment.

Somatic cell hybrids are prepared by fusing somatic cells from different mammals (e.g., human and mouse cells). As hybrids of human and mouse cells grow and divide, they gradually lose human chromosomes in random order, but retain the mouse chromosomes. By using media in which mouse cells cannot grow, because they lack a particular enzyme, but human cells can, the one human chromosome that contains the gene encoding the needed enzyme, will be retained. By using various media, panels of hybrid cell lines can be established. Each cell line in a panel contains either a single human chromosome or a small number of human chromosomes, and a full set of mouse chromosomes, allowing easy mapping of individual genes to specific human chromosomes. (D'Eustachio P. et al. (1983) *Science* 220:919-924). Somatic cell hybrids containing only fragments of human chromosomes can also be produced by using human chromosomes with translocations and deletions.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular sequence to a particular chromosome. Three or more sequences can be assigned per day using a single thermal cycler. Using the STRIFE1 or STRIFE2 nucleotide sequences to design oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes. Other mapping strategies which can similarly be used to map a STRIFE1 or STRIFE2 sequence to its chromosome

include *in situ* hybridization (described in Fan, Y. et al. (1990) *PNAS*, 87:6223-27), pre-screening with labeled flow-sorted chromosomes, and pre-selection by hybridization to chromosome specific cDNA libraries.

Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further be used to provide a precise chromosomal location in one step. Chromosome spreads can be made using cells whose division has been blocked in metaphase by a chemical such as colcemid that disrupts the mitotic spindle. The chromosomes can be treated briefly with trypsin, and then stained with Giemsa. A pattern of light and dark bands develops on each chromosome, so that the chromosomes can be identified individually. The FISH technique can be used with a DNA sequence as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. Preferably 1,000 bases, and more preferably 2,000 bases will suffice to get good results at a reasonable amount of time. For a review of this technique, see Verma *et al.*, Human Chromosomes: A Manual of Basic Techniques (Pergamon Press, New York 1988).

Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. (Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man, available on-line through Johns Hopkins University Welch Medical Library). The relationship between a gene and a disease, mapped to the same chromosomal region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, for example, Egeland, J. *et al.* (1987) *Nature,* 325:783-787.

Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with the STRIFE1 or STRIFE2 gene, can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several

individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

2. Tissue Typing

The STRIFE1 or STRIFE2 sequences of the present invention can also be used to identify individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identification. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The sequences of the present invention are useful as additional DNA markers for RFLP (described in U.S. Patent 5,272,057).

Furthermore, the sequences of the present invention can be used to provide an alternative technique which determines the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the STRIFE1 or STRIFE2 nucleotide sequences described herein can be used to prepare two PCR primers from the 5' and 3' ends of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it.

Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences. The sequences of the present invention can be used to obtain such identification sequences from individuals and from tissue. The STRIFE1 or STRIFE2 nucleotide sequences of the invention uniquely represent portions of the human genome. Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. It is estimated that allelic variation between individual humans occurs with a frequency of about once per each 500 bases. Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. The noncoding sequences of SEQ ID NO:1 or SEQ ID NO:5, can comfortably provide positive individual identification with a panel of perhaps 10 to 1,000 primers which each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences, such as those in SEQ ID NO:3 or SEQ ID NO:7 are used, a more appropriate number of primers for positive individual identification would be 500-2,000.

If a panel of reagents from STRIFE1 or STRIFE2 nucleotide sequences described herein is used to generate a unique identification database for an individual, those same reagents can later be used to identify tissue from that individual. Using the unique identification database, positive identification of the individual, living or dead, can be made from extremely small tissue samples.

3. Use of Partial STRIFE1 or STRIFE2 Sequences in Forensic Biology

DNA-based identification techniques can also be used in forensic biology. Forensic biology is a scientific field employing genetic typing of biological evidence found at a crime scene as a means for positively identifying, for example, a perpetrator of a crime. To make such an identification, PCR technology can be used to amplify DNA sequences taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, or semen found at a crime scene. The amplified sequence can then be compared to a standard, thereby allowing identification of the origin of the biological sample.

The sequences of the present invention can be used to provide polynucleotide reagents, e.g., PCR primers, targeted to specific loci in the human genome, which can enhance the reliability of DNA-based forensic identifications by, for example, providing another "identification marker" (i.e. another DNA sequence that is unique to a particular individual). As mentioned above, actual base sequence information can be used for identification as an accurate alternative to patterns formed by restriction enzyme generated fragments. Sequences targeted to noncoding regions of SEQ ID NOs:1 or SEQ ID NO:5 are particularly appropriate for this use as greater numbers of polymorphisms occur in the noncoding regions, making it easier to differentiate individuals using this technique. Examples of polynucleotide reagents include the STRIFE1 or STRIFE2 nucleotide sequences or portions thereof, e.g., fragments derived from the noncoding regions of SEQ ID NO:1 or SEQ ID NO:5, having a length of at least 20 bases, preferably at least 30 bases.

The STRIFE1 or STRIFE2 nucleotide sequences described herein can further be used to provide polynucleotide reagents, e.g., labeled or labelable probes which can be used in, for example, an *in situ* hybridization technique, to identify a specific tissue, e.g., brain or lung tissue. This can be very useful in cases where a forensic pathologist is presented with a tissue of unknown origin. Panels of such STRIFE1 or STRIFE2 probes can be used to identify tissue by species and/or by organ type.

In a similar fashion, these reagents, e.g., STRIFE1 or STRIFE2 primers or probes can be used to screen tissue culture for contamination (i.e. screen for the presence of a mixture of different types of cells in a culture).

C. **Predictive Medicine:**

The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, and monitoring clinical trails are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the present invention relates to diagnostic assays for determining STRIFE1 or STRIFE2 protein and/or nucleic acid expression as well as STRIFE1 or STRIFE2 activity, in the context of a biological sample (e.g., blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant STRIFE1 or STRIFE2 expression or activity. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with STRIFE1 or STRIFE2 protein, nucleic acid expression or activity. For example, mutations in a STRIFE1 or STRIFE2 gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby phophylactically treat an individual prior to the onset of a disorder characterized by or associated with STRIFE1 or STRIFE2 protein, nucleic acid expression or activity.

Another aspect of the invention pertains to monitoring the influence of agents (e.g., drugs, compounds) on the expression or activity of STRIFE1 or STRIFE2 in clinical trials.

These and other agents are described in further detail in the following sections.

1. **Diagnostic Assays**

An exemplary method for detecting the presence or absence of STRIFE1 or STRIFE2 protein or nucleic acid in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting STRIFE1 or STRIFE2 protein or nucleic acid (e.g., mRNA, genomic DNA) that encodes STRIFE1 or STRIFE2 protein such that the presence of STRIFE1 or STRIFE2 protein or nucleic acid is detected in the biological sample. A preferred agent for detecting STRIFE1 or STRIFE2 mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to STRIFE1 or STRIFE2 mRNA or genomic DNA. The nucleic acid probe can be, for example, a full-length STRIFE1 or STRIFE2 nucleic acid, such as the nucleic acid of SEQ ID NO: 1 or SEQ ID NO:5, or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to STRIFE1 or STRIFE2 mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of the invention are described herein.

A preferred agent for detecting STRIFE1 or STRIFE2 protein is an antibody capable of binding to STRIFE1 or STRIFE2 protein, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (e.g., Fab or $F(ab')_2$) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (i.e., physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect STRIFE1 or STRIFE2 mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of STRIFE1 or STRIFE2 mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of STRIFE1 or STRIFE2 protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. *In vitro* techniques for detection of STRIFE1 or STRIFE2 genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for detection of STRIFE1 or STRIFE2 protein include introducing into a subject a labeled anti-STRIFE1 or STRIFE2 antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

In one embodiment, the biological sample contains protein molecules from the test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a serum sample isolated by conventional means from a subject.

In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting STRIFE1 or STRIFE2 protein, mRNA, or genomic DNA, such that the presence of STRIFE1 or STRIFE2 protein, mRNA or genomic DNA is detected in the biological sample, and comparing the presence of STRIFE1 or STRIFE2 protein, mRNA or genomic DNA in the control sample with the presence of STRIFE1 or STRIFE2 protein, mRNA or genomic DNA in the test sample.

The invention also encompasses kits for detecting the presence of STRIFE1 or STRIFE2 in a biological sample. For example, the kit can comprise a labeled compound or agent capable of detecting STRIFE1 or STRIFE2 protein or mRNA in a biological sample; means for determining the amount of STRIFE1 or STRIFE2 in the sample; and means for comparing the amount of STRIFE1 or STRIFE2 in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect STRIFE1 or STRIFE2 protein or nucleic acid.

2. **Prognostic Assays**

The diagnostic methods described herein can furthermore be utilized to identify subjects having or at risk of developing a disease or disorder associated with aberrant STRIFE1 or STRIFE2 expression or activity. For example, the assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a disorder associated with STRIFE1 or STRIFE2 protein, nucleic acid expression or activity such as a TNF-associated disorder, e.g., inflammatory, immune, or neoplastic disorder. Thus, the present invention provides a method for identifying a disease or disorder associated with aberrant STRIFE1 or STRIFE2 expression or activity in which a test sample is obtained from a subject and STRIFE1 or STRIFE2 protein or nucleic acid (e.g, mRNA, genomic DNA) is detected, wherein the presence of STRIFE1 or STRIFE2 protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant STRIFE1 or STRIFE2 expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (e.g., serum), cell sample, or tissue.

Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (e.g., an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant STRIFE1 or STRIFE2 expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent for a disorder, such as a proliferative disorder, a differentiative or developmental disorder, or a hematopoietic disorder. Alternatively, such methods can be used to determine whether a subject can be effectively treated with an agent for a differentiative or proliferative disease (e.g., cancer). Thus, the present invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder associated with aberrant STRIFE1 or STRIFE2 expression or activity in which a test sample is obtained and STRIFE1 or STRIFE2 protein or

nucleic acid expression or activity is detected (e.g., wherein the abundance of STRIFE1 or STRIFE2 protein or nucleic acid expression or activity is diagnostic for a subject that can be administered the agent to treat a disorder associated with aberrant STRIFE1 or STRIFE2 expression or activity.)

The methods of the invention can also be used to detect genetic alterations in an STRIFE1 or STRIFE2 gene, thereby determining if a subject with the altered gene is at risk for a disorder characterized by aberrant development, aberrant cellular differentiation, aberrant cellular proliferation or an aberrant hematopoietic response. In preferred embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic alteration characterized by at least one of an alteration affecting the integrity of a gene encoding a STRIFE1 or STRIFE2-protein, or the mis-expression of the STRIFE1 or STRIFE2 gene. For example, such genetic alterations can be detected by ascertaining the existence of at least one of 1) a deletion of one or more nucleotides from an STRIFE1 or STRIFE2 gene; 2) an addition of one or more nucleotides to a STRIFE1 or STRIFE2 gene; 3) a substitution of one or more nucleotides of a STRIFE1 or STRIFE2 gene, 4) a chromosomal rearrangement of a STRIFE1 or STRIFE2 gene; 5) an alteration in the level of a messenger RNA transcript of a STRIFE1 or STRIFE2 gene, 6) aberrant modification of a STRIFE1 or STRIFE2 gene, such as of the methylation pattern of the genomic DNA, 7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of a STRIFE1 or STRIFE2 gene, 8) a non-wild type level of a STRIFE1 or STRIFE2-protein, 9) allelic loss of a STRIFE1 or STRIFE2 gene, and 10) inappropriate post-translational modification of a STRIFE1 or STRIFE2-protein. As described herein, there are a large number of assay techniques known in the art which can be used for detecting alterations in a STRIFE1 or STRIFE2 gene. A preferred biological sample is a tissue or serum sample isolated by conventional means from a subject.

In certain embodiments, detection of the alteration involves the use of a probe/primer in a polymerase chain reaction (PCR) (see, e.g., U.S. Patent Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (see, e.g., Landegran *et al.* (1988) *Science* 241:1077-1080; and Nakazawa *et al.* (1994) *PNAS* 91:360-364), the latter of which can be particularly useful for detecting point mutations in the STRIFE1 or STRIFE2-gene (see Abravaya *et al.* (1995) *Nucleic Acids Res* .23:675-682). This method can include the steps of collecting a sample of cells from a patient, isolating nucleic acid (e.g., genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers which specifically hybridize to a STRIFE1 or STRIFE2 gene under conditions such that hybridization and amplification of the STRIFE1 or STRIFE2-gene

(if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (Guatelli, J.C. *et al.*, 1990, Proc. Natl. Acad. Sci. USA 87:1874-1878), transcriptional amplification system (Kwoh, D.Y. *et al.*, 1989, Proc. Natl. Acad. Sci. USA 86:1173-1177), Q-Beta Replicase (Lizardi, P.M. et all, 1988, Bio/Technology 6:1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in a STRIFE1 or STRIFE2 gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (see, for example, U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in STRIFE1 or STRIFE2 can be identified by hybridizing a sample and control nucleic acids, e.g., DNA or RNA, to high density arrays containing hundreds or thousands of oligonucleotides probes (Cronin, M.T. *et al.* (1996) *Human Mutation* 7: 244-255; Kozal, M.J. *et al.* (1996) *Nature Medicine* 2: 753-759). For example, genetic mutations in STRIFE1 or STRIFE2 can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin, M.T. *et al. supra.* Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential ovelapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the STRIFE1 or STRIFE2 gene and detect mutations by comparing the sequence of the sample STRIFE1 or STRIFE2 with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxim and Gilbert ((1977) *PNAS* 74:560) or Sanger ((1977) *PNAS* 74:5463). It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays ((1995) *Biotechniques* 19:448), including sequencing by mass spectrometry (see, e.g., PCT International Publication No. WO 94/16101; Cohen *et al.* (1996) *Adv. Chromatogr.* 36:127-162; and Griffin *et al.* (1993) *Appl. Biochem. Biotechnol.* 38:147-159).

Other methods for detecting mutations in the STRIFE1 or STRIFE2 gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers *et al.* (1985) *Science* 230:1242). In general, the art technique of "mismatch cleavage" starts by providing heteroduplexes of formed by hybridizing (labeled) RNA or DNA containing the wild-type STRIFE1 or STRIFE2 sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent which cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA hybrids treated with S1 nuclease to enzymatically digesting the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. See, for example, Cotton *et al.* (1988) *Proc. Natl Acad Sci USA* 85:4397; Saleeba *et al.* (1992) *Methods Enzymol.* 217:286-295. In a preferred embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in STRIFE1 or STRIFE2 cDNAs obtained from samples of cells. For example, the mutY enzyme of E. coli cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu *et al.* (1994) *Carcinogenesis* 15:1657-1662). According to an exemplary embodiment, a probe based on an STRIFE1 or STRIFE2 sequence, e.g., a wild-type STRIFE1 or STRIFE2 sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be

detected from electrophoresis protocols or the like. See, for example, U.S. Patent No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in STRIFE1 or STRIFE2 genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita *et al.* (1989) *Proc Natl. Acad. Sci USA*: 86:2766, see also Cotton (1993) *Mutat Res* 285:125-144; and Hayashi (1992) *Genet Anal Tech Appl* 9:73-79). Single-stranded DNA fragments of sample and control STRIFE1 or STRIFE2 nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In a preferred embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen *et al.* (1991) *Trends Genet* 7:5).

In yet another embodiment the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers *et al.* (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner (1987) *Biophys Chem* 265:12753).

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions which permit hybridization only if a perfect match is found (Saiki *et al.* (1986) *Nature* 324:163); Saiki *et al.* (1989) *Proc. Natl Acad. Sci USA* 86:6230). Such allele specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele specific amplification technology which depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of

interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs *et al.* (1989) *Nucleic Acids Res.* 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini *et al.* (1992) *Mol. Cell Probes* 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification (Barany (1991) *Proc. Natl. Acad. Sci USA* 88:189). In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, *e.g.*, in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving a STRIFE1 or STRIFE2 gene.

Furthermore, any cell type or tissue in which STRIFE1 or STRIFE2 is expressed may be utilized in the prognostic assays described herein.

3. Monitoring of Effects During Clinical Trials

Monitoring the influence of agents (e.g., drugs, compounds) on the expression or activity of STRIFE1 or STRIFE2 (e.g., modulation of cellular signal transduction, regulation of gene transcription in a cell involved in development or differentiation, regulation of cellular proliferation) can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase STRIFE1 or STRIFE2 gene expression, protein levels, or upregulate STRIFE1 or STRIFE2 activity, can be monitored in clinical trails of subjects exhibiting decreased STRIFE1 or STRIFE2 gene expression, protein levels, or downregulated STRIFE1 or STRIFE2 activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease STRIFE1 or STRIFE2 gene expression, protein levels, or downregulate STRIFE1 or STRIFE2 activity, can be monitored in clinical trails of subjects exhibiting increased STRIFE1 or STRIFE2 gene expression, protein levels, or upregulated STRIFE1 or STRIFE2 activity. In such clinical trials, the expression or activity of STRIFE1 or STRIFE2 and, preferably, other genes that have been implicated in, for example, a proliferative disorder can be used as a "read out" or markers of the phenotype of a particular cell.

For example, and not by way of limitation, genes, including STRIFE1 or STRIFE2, that are modulated in cells by treatment with an agent (e.g., compound, drug or small molecule) which modulates STRIFE1 or STRIFE2 activity (e.g., identified in a screening assay as described herein) can be identified. Thus, to study the effect of agents on proliferative disorders, developmental or differentiative disorder, or hematopoietic disorder, for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of STRIFE1 or STRIFE2 and other genes implicated in the proliferative disorder, developmental or differentiative disorder, or hematopoietic disorder, respectively. The levels of gene expression (i.e., a gene expression pattern) can be quantified by Northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein produced, by one of the methods as described herein, or by measuring the levels of activity of STRIFE1 or STRIFE2 or other genes. In this way, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during treatment of the individual with the agent.

In a preferred embodiment, the present invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (e.g., an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) comprising the steps of (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting the level of expression of a STRIFE1 or STRIFE2 protein, mRNA, or genomic DNA in the preadministration sample; (iii) obtaining one or more post-administration samples from the subject; (iv) detecting the level of expression or activity of the STRIFE1 or STRIFE2 protein, mRNA, or genomic DNA in the post-administration samples; (v) comparing the level of expression or activity of the STRIFE1 or STRIFE2 protein, mRNA, or genomic DNA in the pre-administration sample with the STRIFE1 or STRIFE2 protein, mRNA, or genomic DNA in the post administration sample or samples; and (vi) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of STRIFE1 or STRIFE2 to higher levels than detected, i.e., to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of STRIFE1 or STRIFE2 to lower levels than detected, i.e. to decrease the effectiveness of the agent. According to such an embodiment, STRIFE1 or STRIFE2 expression or activity may be used as an indicator of the effectiveness of an agent, even in the absence of an observable phenotypic response.

C. **Methods of Treatment:**

The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant STRIFE1 or STRIFE2 expression or activity. With regards to both prophylactic and therapeutic methods of treatment, such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics. "Pharmacogenomics", as used herein, refers to the application of genomics technologies such as gene sequencing, statistical genetics, and gene expression analysis to drugs in clinical development and on the market. More specifically, the term refers to the study of how a patient's genes determine his or her response to a drug (e.g., a patient's "drug response phenotype", or "drug response genotype"). Thus, another aspect of the invention provides methods for tailoring an individual's prophylactic or therapeutic treatment with either the STRIFE1 or STRIFE2 molecules of the present invention or STRIFE1 or STRIFE2 modulators according to that individual's drug response genotype. Pharmacogenomics allows a clinician or physician to target prophylactic or therapeutic treatments to patients who will most benefit from the treatment and to avoid treatment of patients who will experience toxic drug-related side effects.

1. **Prophylactic Methods**

In one aspect, the invention provides a method for preventing in a subject, a disease or condition associated with an aberrant STRIFE1 or STRIFE2 expression or activity, by administering to the subject an agent which modulates STRIFE1 or STRIFE2 expression or at least one STRIFE1 or STRIFE2 activity. Subjects at risk for a disease which is caused or contributed to by aberrant STRIFE1 or STRIFE2 expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the STRIFE1 or STRIFE2 aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of STRIFE1 or STRIFE2 aberrancy, for example, an STRIFE1 or STRIFE2 agonist or STRIFE1 or STRIFE2 antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein. The prophylactic methods of the present invention are further discussed in the following subsections.

2. **Therapeutic Methods**

Another aspect of the invention pertains to methods of modulating STRIFE1 or STRIFE2 expression or activity for therapeutic purposes. The modulatory method of the invention involves contacting a cell with an agent that modulates one or more of the activities of STRIFE1 or STRIFE2 protein activity associated with the cell. An agent that modulates STRIFE1 or STRIFE2 protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring target molecule of a STRIFE1 or STRIFE2 protein, a peptide, a STRIFE1 or STRIFE2 peptidomimetic, or other small molecule. In one embodiment, the agent stimulates one or more STRIFE1 or STRIFE2 protein activity. Examples of such stimulatory agents include active STRIFE1 or STRIFE2 protein and a nucleic acid molecule encoding STRIFE1 or STRIFE2 that has been introduced into the cell. In another embodiment, the agent inhibits one or more STRIFE1 or STRIFE2 protein activity. Examples of such inhibitory agents include antisense STRIFE1 or STRIFE2 nucleic acid molecules and anti-STRIFE1 or STRIFE2 antibodies. These modulatory methods can be performed *in vitro* (e.g., by culturing the cell with the agent) or, alternatively, *in vivo* (e.g, by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant expression or activity of a STRIFE1 or STRIFE2 protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (e.g., an agent identified by a screening assay described herein), or combination of agents that modulates (e.g., upregulates or downregulates) STRIFE1 or STRIFE2 expression or activity. In another embodiment, the method involves administering a STRIFE1 or STRIFE2 protein or nucleic acid molecule as therapy to compensate for reduced or aberrant STRIFE1 or STRIFE2 expression or activity.

Stimulation of STRIFE1 or STRIFE2 activity is desirable in situations in which STRIFE1 or STRIFE2 is abnormally downregulated and/or in which increased STRIFE1 or STRIFE2 activity is likely to have a beneficial effect. Likewise, inhibition of STRIFE1 or STRIFE2 activity is desirable in situations in which STRIFE1 or STRIFE2 is abnormally upregulated and/or in which decreased STRIFE1 or STRIFE2 activity is likely to have a beneficial effect. One example of such a situation is where a subject has a TNF-associated disorder, e.g., an inflammatory, immune, or neoplastic disorder.

3. **Pharmacogenomics**

The STRIFE1 or STRIFE2 molecules of the present invention, as well as agents, or modulators which have a stimulatory or inhibitory effect on STRIFE1 or STRIFE2 activity (e.g., STRIFE1 or STRIFE2 gene expression) as identified by a screening assay

described herein can be administered to individuals to treat (prophylactically or therapeutically) disorders (e.g, TNF-associated disorders) associated with aberrant STRIFE1 or STRIFE2 activity. In conjunction with such treatment, pharmacogenomics (i.e., the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, a physician or clinician may consider applying knowledge obtained in relevant pharmacogenomics studies in determining whether to administer an STRIFE1 or STRIFE2 molecule or STRIFE1 or STRIFE2 modulator as well as tailoring the dosage and/or therapeutic regimen of treatment with an STRIFE1 or STRIFE2 molecule or STRIFE1 or STRIFE2 modulator.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See e.g., Eichelbaum, M., *Clin Exp Pharmacol Physiol*, 1996, 23(10-11) :983-985 and Linder, M.W., *Clin Chem*, 1997, 43(2):254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare genetic defects or as naturally-occurring polymorphisms. For example, glucose-6-phosphate dehydrogenase deficiency (G6PD) is a common inherited enzymopathy in which the main clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

One pharmacogenomics approach to identifying genes that predict drug response, known as "a genome-wide association", relies primarily on a high-resolution map of the human genome consisting of already known gene-related markers (e.g., a "bi-allelic" gene marker map which consists of 60,000-100,000 polymorphic or variable sites on the human genome, each of which has two variants). Such a high-resolution genetic map can be compared to a map of the genome of each of a statistically significant number of patients taking part in a Phase II/III drug trial to identify markers associated with a particular observed drug response or side effect. Alternatively, such a high resolution map can be generated from a combination of some ten-million known single nucleotide polymorphisms (SNPs) in the human genome. As used herein, a "SNP" is a common alteration that occurs in a single nucleotide base in a stretch of DNA. For example, a SNP may occur once per every 1000 bases of DNA. A SNP may be involved in a disease process, however, the vast majority may not be disease-

associated. Given a genetic map based on the occurrence of such SNPs, individuals can be grouped into genetic categories depending on a particular pattern of SNPs in their individual genome. In such a manner, treatment regimens can be tailored to groups of genetically similar individuals, taking into account traits that may be common among such genetically similar individuals.

Alternatively, a method termed the "candidate gene approach", can be utilized to identify genes that predict drug response. According to this method, if a gene that encodes a drug target is known (e.g., a STRIFE1 or STRIFE2 protein or a STRIFE1 receptor of the present invention), all common variants of that gene can be identified in the population and a particular drug response can be associated with one or more genes.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (e.g., N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. The other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

Alternatively, a method termed the "gene expression profiling", can be utilized to identify genes that predict drug response. For example, the gene expression of an animal dosed with a drug (e.g., a STRIFE1 or STRIFE2 molecule or STRIFE1 or STRIFE2 modulator of the present invention) can give an indication whether gene pathways related to toxicity have been turned on.

Information generated from more than one of the above pharmacogenomics approaches can be used to determine appropriate dosage and treatment regimens for prophylactic or therapeutic treatment an individual. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with a STRIFE1

or STRIFE2 molecule or STRIFE1 or STRIFE2 modulator, such as a modulator identified by one of the exemplary screening assays described herein.

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application are incorporated herein by reference.

EXAMPLES

EXAMPLE 1: IDENTIFICATION OF MURINE STRIFE1 AND STRIFE2 cDNA

In this example, the isolation and characterization of the cDNA encoding murine STRIFE1 and STRIFE2 is described. STRIFE is a mouse gene which encodes a protein belonging to the TNFR family. Two splice forms have been identified, one that is predicted to be membrane bound (STRIFE1) and one that is secreted (STRIFE2).

STRIFE was identified as a TNFR homologue by a computer-based search of the public EST databases. More specifically, the murine STRIFE1 and STRIFE2 cDNA were identified by searching against a copy of the GenBank nucleotide database using the BLASTN™ program (BLASTN 1.3MP: Altschul et al., *J. Mol. Bio.* 215:403, 1990). Numerous clones that consisted mostly of 3' reads and some that were 5' reads within the 3' untranslated region were found by this search. The sequences were analyzed against a non-redundant protein database with the BLASTX™ program, which translates a nucleic acid sequence in all six frames and compares it against available protein databases (BLASTX 1.3MP:Altschul et al., *supra*). This protein database is a combination of the Swiss-Prot, PIR, and NCBI GenPept protein databases. Two clones (Accession Numbers AA036247 and AA003356) were obtained from the IMAGE consortium, and fully sequenced. The additional sequencing of AA036247 (T 127a; STRIFE1) extended the original EST by 623 nucleotides (see SEQ ID NO:1) and the further sequencing of AA003356 (T127b; STRIFE2) extended the original EST by 254 nucleotides (see SEQ ID NO:5).

A BLASTN™ search of the EST database revealed the following ESTs having significant homology to clone Accession Number AA036247:

EST Database hits	Species	Base Pairs Covered	% Identity	Coding?
Accession # AA495217	zebrafish	602-711	82	yes

A BLASTN™ search of the EST and nucleotide database revealed the following ESTs and nucleotides having significant homology to clone Accession Number AA003356:

EST Database hits	Species	Base Pairs Covered	% Identity	Coding?
Accession # AA686080	rat	297-367	64	yes
Accession # AA209382	human	150-210	67	yes
Accession # AA409240	mouse	284-319	80	yes
Accession # N91779	mouse	519-489	83	yes

EXAMPLE 2: TISSUE EXPRESSION OF THE STRIFE1 AND STRIFE2 GENE

Human I and mouse multiple tissue northern (MTN) blots (Clontech, Palo Alto, CA) containing 2 μg of poly A+ RNA per lane were probed with a 750bp Eco*RI*/Not*I* fragment of the mouse STRIFE1 cDNA. The filters were prehybridized in 10 ml of Express Hyb hybridization solution (Clontech, Palo Alto, CA) at 68°C for 1 hour, after which 100 ng of ^{32}P labeled probe was added. The probe was generated using the Stratagene Prime-It kit, Catalog Number 300392 (Clontech, Palo Alto, CA). Hybridization was allowed to proceed at 68°C for approximately 2 hours. The filters were washed in a 0.05% SDS/2X SSC solution for 15 minutes at room temperature and then twice with a 0.1% SDS/0.1X SSC solution for 20 minutes at 50°C and then exposed to autoradiography film overnight at -80°C with one screen. The mouse tissues tested included: heart, brain, spleen, lung, liver, skeletal muscle, kidney, and testis. The human tissues tested included: heart, placenta, lung, liver, skeletal muscle, kidney, and pancreas.

There was a strong hybridization to both mouse and human heart, brain, and lung indicating that the approximately 4.4 kb STRIFE1 and STRIFE2 gene transcript is expressed in these tissues.

EXAMPLE 3: EXPRESSION OF RECOMBINANT STRIFE1 AND STRIFE2 PROTEIN IN BACTERIAL CELLS

In this example, STRIFE1 or STRIFE2 is expressed as a recombinant glutathione-S-transferase (GST) fusion polypeptide in *E. coli* and the fusion polypeptide is isolated and characterized. Specifically, STRIFE1 or STRIFE2 is fused to GST and this fusion polypeptide is expressed in *E. coli*, e.g., strain PEB199. As the murine STRIFE1 and STRIFE2 proteins are predicted to be approximately 23.55 kDa and 16.72 kDa, respectively, and GST is predicted to be 26 kDa, the fusion polypeptides are predicted to be approximately 49.55 kDa and 42.72 kDa, respectively, in molecular weight. Expression of the GST-STRIFE1 or STRIFE2 fusion protein in PEB199 is induced with IPTG. The recombinant fusion polypeptide is purified from crude bacterial

lysates of the induced PEB199 strain by affinity chromatography on glutathione beads. Using polyacrylamide gel electrophoretic analysis of the polypeptide purified from the bacterial lysates, the molecular weight of the resultant fusion polypeptide is determined.

EXAMPLE 4: EXPRESSION OF RECOMBINANT STRIFE1 AND STRIFE2 PROTEIN IN COS CELLS

To express the STRIFE1 or STRIFE2 gene in COS cells, the pcDNA/Amp vector by Invitrogen Corporation (San Diego, CA) is used. This vector contains an SV40 origin of replication, an ampicillin resistance gene, an *E. coli* replication origin, a CMV promoter followed by a polylinker region, and an SV40 intron and polyadenylation site. A DNA fragment encoding the entire STRIFE1 or STRIFE2 protein and a HA tag (Wilson et al. (1984) *Cell* 37:767) fused in-frame to its 3' end of the fragment is cloned into the polylinker region of the vector, thereby placing the expression of the recombinant protein under the control of the CMV promoter.

To construct the plasmid, the STRIFE1 or STRIFE2 DNA sequence is amplified by PCR using two primers. The 5' primer contains the restriction site of interest followed by approximately twenty nucleotides of the STRIFE1 or STRIFE2 coding sequence starting from the initiation codon; the 3' end sequence contains complementary sequences to the other restriction site of interest, a translation stop codon, the HA tag and the last 20 nucleotides of the STRIFE1 or STRIFE2 coding sequence. The PCR amplified fragment and the pCDNA/Amp vector are digested with the appropriate restriction enzymes and the vector is dephosphorylated using the CIAP enzyme (New England Biolabs, Beverly, MA). Preferably the two restriction sites chosen are different so that the STRIFE1 or STRIFE2 gene is inserted in the correct orientation. The ligation mixture is transformed into *E. coli* cells (strains HB101, DH5a, SURE, available from Stratagene Cloning Systems, La Jolla, CA, can be used), the transformed culture is plated on ampicillin media plates, and resistant colonies are selected. Plasmid DNA is isolated from transformants and examined by restriction analysis for the presence of the correct fragment.

COS cells are subsequently transfected with the STRIFE1 or STRIFE2-pcDNA/Amp plasmid DNA using the calcium phosphate or calcium chloride co-precipitation methods, DEAE-dextran-mediated transfection, lipofection, or electroporation. Other suitable methods for transfecting host cells can be found in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989. The expression of the STRIFE1 and STRIFE2 polypeptide is detected by radiolabelling (^{35}S-methionine or ^{35}S-cysteine available from NEN, Boston,

MA, can be used) and immunoprecipitation (Harlow, E. and Lane, D. *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988) using an HA specific monoclonal antibody. Briefly, the cells are labelled for 8 hours with ^{35}S-methionine (or ^{35}S-cysteine). The culture media are then collected and the cells are lysed using detergents (RIPA buffer, 150 mM NaCl, 1% NP-40, 0.1% SDS, 0.5% DOC, 50 mM Tris, pH 7.5). Both the cell lysate and the culture media are precipitated with an HA specific monoclonal antibody. Precipitated polypeptides are then analyzed by SDS-PAGE.

Alternatively, DNA containing the STRIFE1 or STRIFE2 coding sequence is cloned directly into the polylinker of the pCDNA/Amp vector using the appropriate restriction sites. The resulting plasmid is transfected into COS cells in the manner described above, and the expression of the STRIFE1 or STRIFE2 polypeptide is detected by radiolabelling and immunoprecipitation using an STRIFE1 or STRIFE2 specific monoclonal antibody.

EXAMPLE 5: CHARACTERIZATION OF THE MURINE STRIFE1 AND STRIFE2 PROTEINS

STRIFE1 is approximately 981 nucleotides in length and has an open reading frame of 645 nucleotides that is predicted to encode a protein of 214 amino acids. STRIFE2 is approximately 655 nucleotides long with an open reading frame of 453 nucleotides predicted to encode a protein of 150 amino acids. Both clones have been subcloned into a variety of expression vectors including those for retroviral delivery and for expression in bacterial, yeast and mammalian cells.

BlastX searching of the protein database confirms the homology of this clone to various members of the TNFR family. The extracellular domains of STRIFE1 and STRIFE2 are approximately 40% identical to OX40. Importantly, a number of cysteine residues within the extracellular domains of STRIFE1 and STRIFE2 match the cysteine-rich domain signature of the TNFR/NGFR family (Prosite Accession PDOC00561). The program SignalP (Nielsen et al, 1997) predicts a 30 amino acid signal peptide at the very N-terminus of both STRIFE1 and STRIFE2 (i.e., aa 1-29 of SEQ ID NOs:1 and 5). The predicted molecular weight for STRIFE1 is approximately 23.55 kDa with the signal peptide and 20.34 kDa without the signal peptide which is presumed to be cleaved in the mature protein. There are no obvious motifs in the small intracellular domain of STRIFE1. STRIFE2 is predicted to be 16.72 kDa with the signal peptide and 13.51 kDa without the signal peptide.

A FASTA search (described in Pearson W.R. & Lipman D.J. (1988) *PNAS* 85:2444-2448, score matrix: PAM120) using the STRIFE1 protein sequence as a query,

indicates that STRIFE1 is 85.7% identical to the human OAF065 receptor (Accession number W70387; described in PCT application number WO 98/38304, published on September 3, 1998) over amino acid residues 1-203. The results from this search are shown in Figure 4.

A FASTA search (described in Pearson W.R. & Lipman D.J. (1988) *PNAS* 85:2444-2448, score matrix: PAM120) using the STRIFE1 nucleotide sequence as a query, indicates that STRIFE1 is 70.6% identical to the nucleic acid molecule encoding the human OAF065 receptor (Accession number V33362; described in PCT application number WO 98/38304, published on September 3, 1998) over nucleotide residues 65-981. The results from this search are shown in Figure 5.

Structure of the STRIFE1 and STRIFE2 Family proteins

An alignment of the amino acid sequences of murine STRIFE1, STRIFE2, and murine OX40 (Accesssion Number P47741) is shown in Figure 3. Amino acid residues which are conserved between murine STRIFE1 and STRIFE2 family members are highlighted. The percent identity was calculated using the alignment generated using MegAlign™ sequence alignment software. The initial pairwise alignment step was performed using a Wilbur-Lipmann algorithm with a K-tuple of 2, a GAP penalty of 5, a window of 4, and diagonals saved set to = 4. The multiple alignment step was performed using the Clustal algorithm with a PAM 250 residue weight Table, a GAP penalty of 10, and a GAP length penalty of 10.

Retroviral Delivery of STRIFE1 and STRIFE2 into mice

The entire open reading frame of STRIFE1 or STRIFE2 is subcloned into the retroviral vector MSCVneo, described in Hawley et al.(1994) *Gene Therapy* 1:136-138. Cells (293Ebna, Invitrogen) are then transiently transfected with the STRIFE1 or STRIFE2 construct and with constructs containing viral regulatory elements, to produce high titre retrovirus containing the STRIFE1 or STRIFE2 gene. This virus is then used to transfect mice. These mice are then tested for any gross pathology and for changes in their immune response using standard assays.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

What is claimed:

1. An isolated nucleic acid molecule which encodes a STRIFE protein, comprising a nucleotide sequence at least about 60% homologous to a nucleotide sequence of SEQ ID NO:3 or SEQ ID NO:7, or a complement thereof.

2. The isolated nucleic acid molecule of claim 1 comprising the nucleotide sequence of SEQ ID NO:3 or a complement thereof.

3. The isolated nucleic acid molecule of claim 2, further comprising nucleotides 1-106 of SEQ ID NO:1.

4. The isolated nucleic acid molecule of claim 2, further comprising nucleotides 752-981 of SEQ ID NO:1.

5. The isolated nucleic acid molecule of claim 1 comprising the nucleotide sequence of SEQ ID NO:7 or a complement thereof.

6. The isolated nucleic acid molecule of claim 5, further comprising nucleotides 1-109 of SEQ ID NO:5.

7. The isolated nucleic acid molecule of claim 5, further comprising nucleotides 563-655 of SEQ ID NO:5.

8. The isolated nucleic acid molecule of either of claims 1 or 7 which specifically detects a STRIFE nucleic acid molecule encoding a STRIFE protein relative to a nucleic acid molecule encoding a non-STRIFE protein.

9. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a protein which comprises an amino acid sequence at least about 60% homologous to the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:6.

10. The isolated nucleic acid molecule of claim 9 comprising a nucleotide sequence encoding a protein which comprises the amino acid sequence of SEQ ID NO:2.

11. The isolated nucleic acid molecule of claim 9 comprising a nucleotide sequence encoding a protein which comprises the amino acid sequence of SEQ ID NO:6.

12. An isolated nucleic acid molecule encoding a STRIFE protein, comprising a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:5.

13. An isolated nucleic acid molecule encoding a STRIFE protein, comprising a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:3 or SEQ ID NO:7.

14. An isolated nucleic acid molecule encoding a STRIFE protein, comprising a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:4 or SEQ ID NO:8.

15. An isolated nucleic acid molecule comprising a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising nucleotides 107-751, 1-16, 413-602, or 711-981 of SEQ ID NO:1.

16. An isolated nucleic acid molecule comprising a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising nucleotides 110-562, 1-16, 416-489, or 519-655 of SEQ ID NO:5.

17. An isolated nucleic acid molecule at least 450 nucleotides in length which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:5, SEQ ID NO:4 or SEQ ID NO:8.

18. An isolated nucleic acid molecule which is antisense to the nucleic acid molecule of any of claims 1, 10, 11, 13, 14, or 15.

19. A vector comprising the nucleic acid molecule of any of claims 1, 9, 12, 13, or 14.

20. The vector of claim 19, which is a recombinant expression vector.

21. A host cell containing the vector of claim 20.

22. A method for producing STRIFE protein comprising culturing the host cell of claim 21 in a suitable medium until STRIFE protein is produced.

23. The method of claim 22, further comprising isolating STRIFE protein from the medium or the host cell.

24. A nonhuman transgenic animal which contains cells carrying a transgene encoding STRIFE protein.

25. A nonhuman homologous recombinant animal which contains cells having an altered STRIFE gene.

26. An isolated STRIFE protein comprising an amino acid sequence at least about 60% homologous to the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:6.

27. An isolated STRIFE protein which is encoded by a nucleic acid molecule comprising a nucleotide sequence at least about 60% homologous to a nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID , or a complement thereof.

28. An isolated STRIFE protein which is encoded by a nucleic acid molecule comprising a nucleotide sequence at least about 60% homologous to a nucleotide sequence of SEQ ID NO:3 or SEQ ID NO:7, or a complement thereof.

29. An isolated STRIFE protein which is encoded by a nucleic acid molecule comprising a nucleotide sequence at least about 60% homologous to a nucleotide sequence of SEQ ID NO:4 or SEQ ID NO:8, or a complement thereof.

30. An isolated STRIFE protein which is encoded by a nucleic acid molecule comprising a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:8.

31. An isolated protein comprising an amino acid sequence sufficiently homologous to the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:6, wherein the protein retains a STRIFE biological activity.

32. The isolated protein of claim 31 comprising an amino acid sequence 60% homologous to an amino acid sequence of SEQ ID NO:2.

33. The isolated protein of claim 31 comprising an amino acid sequence 60% homologous to an amino acid sequence of SEQ ID NO:6.

34. The isolated protein of any of claims 26-33, comprising a signal sequence.

35. The isolated protein of any of claims 26-33, comprising an N-terminal cysteine-rich domain.

36. An isolated protein comprising the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:6.

37. A fusion protein comprising a STRIFE polypeptide operatively linked to a non-STRIFE polypeptide.

38. The fusion protein of claim 37, wherein the non-STRIFE polypeptide comprises a signal sequence.

39. The fusion protein of claim 37, wherein the non-STRIFE polypeptide is an immunoglobulin domain.

40. An antibody that specifically binds STRIFE.

41. The antibody of claim 40, which is monoclonal.

42. The antibody of claim 41, which is labeled with a detectable substance.

43. A pharmaceutical composition comprising the protein of any one of claims 26-33, or 37, and a pharmaceutically acceptable carrier.

44. A pharmaceutical composition comprising the antibody of claim 40 and a pharmaceutically acceptable carrier.

45. A method for modulating a cell-associated activity comprising contacting a cell with an agent which modulates STRIFE protein activity or STRIFE nucleic acid expression such that the cell-associated activity is altered relative to the cell-associated activity of the cell in the absence of the agent.

46. The method of claim 45, wherein the agent stimulates a STRIFE protein activity or expression.

47. The method of claim 45, wherein the agent inhibits a STRIFE protein activity or expression.

48. The method of claim 47, wherein the agent is an antisense STRIFE nucleic acid molecule.

49. The method of claim 47, wherein the agent is an antibody that specifically binds to STRIFE.

50. The method of claim 45, wherein the cell is present within a subject and the agent is administered to the subject.

51. A method for treating a subject having a disorder characterized by aberrant STRIFE protein activity or nucleic acid expression comprising administering to the subject a STRIFE modulator such that treatment of the subject occurs.

52. The method of claim 51, wherein the STRIFE modulator is a small molecule.

53. The method of claim 51, wherein the STRIFE modulator is a STRIFE protein.

54. The method of claim 51 wherein the STRIFE modulator is a nucleic acid molecule encoding a STRIFE protein.

55. The method of claim 51, wherein the disorder is a differentiative disorder.

56. The method of claim 51, wherein the disorder is a proliferative disorder.

57. A method for detecting the presence of STRIFE activity in a biological sample comprising contacting a biological sample with an agent capable of detecting an indicator of STRIFE activity such that the presence of STRIFE activity is detected in the biological sample.

58. The method of claim 57, wherein the agent detects STRIFE mRNA.

59. The method of claim 57, wherein the agent is a labeled nucleic acid probe capable of hybridizing to STRIFE mRNA.

60. The method of claim 57, wherein the agent detects STRIFE protein.

61. The method of claim 57, wherein the agent is a labeled antibody capable of specifically binding to STRIFE protein.

62. A kit for detecting the presence of STRIFE activity in a biological sample comprising an agent capable of detecting an indicator of STRIFE activity in a biological sample.

63. The kit of claim 62, wherein the agent is a nucleic acid probe capable of hybridizing to STRIFE mRNA.

64. The kit of claim 62, wherein the agent is an antibody capable of specifically binding to STRIFE protein.

65. The kit of claim 62, further comprising instructions for use.

66. A diagnostic assay for identifying a genetic alteration in a cell sample, the presence or absence of the genetic alteration characterized by at least one of (i) aberrant modification or mutation of a gene encoding a STRIFE protein, and (ii) mis-regulation of said gene or (iii) aberrant post-translational modification of a STRIFE protein.

67. The assay of claim 66, wherein detecting said alteration includes:

a. providing a reagent comprising a diagnostic probe of claim 15, 16, 17 or 18;

b. combining said reagent with nucleic acid of said cell sample; and

c. detecting, by hybridization of said probe to said cellular nucleic acid, the existence of at least one of a deletion of one or more nucleotides from said gene, an addition of one or more nucleotides to said gene, a substitution of one or more nucleotides of said gene, a gross chromosomal rearrangement of all or a portion of said gene, a gross alteration in the level of an mRNA transcript of said gene, or a non-wild type splicing pattern of an mRNA transcript of said gene.

68. The assay of claim 66, wherein detecting said alteration includes:

a. providing a reagent comprising two diagnostic probes;

b. combining said reagent with nucleic acid of said cell sample; and

c. detecting, by amplification or lack of amplification of said cellular nucleic acid, the absence or existence of said alteration.

69. A method for identifying a compound that modulates the activity of a STRIFE protein, comprising:

a. providing a indicator composition comprising a protein having STRIFE activity;

b. contacting the indicator composition with a test compound; and

c. determining the effect of the test compound on STRIFE activity in the indicator composition to thereby identify a compound that modulates the activity of a STRIFE protein.

FIG.1A

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GAATTCGGCACGAGGGCCGGCACCCCCGCGCCACCCCAGCCTCAAACTGCAGTCCGGCGCCGCGGGGCAGGACAAGGGG   79

                                  M   A   L   K   V   L   P   L   H   R   T   V   L    13
AAGGAATAAACACGTTTGGTGAGAGCC ATG GCA CTC AAG GTC CTA CCT CTA CAC AGG ACG GTG CTC   145

 F   A   A   I   L   F   L   L   H   L   A   C   K   V   S   C   E   T   G   D    33
TTC GCT GCC ATT CTC TTC CTA CTC CAC CTG GCA TGT AAA GTG AGT TGC GAA ACC GGA GAT   205

 C   R   Q   Q   E   F   K   D   R   S   G   N   C   V   L   C   K   Q   C   G    53
TGC AGG CAG CAG GAA TTC AAG GAT CGA TCT GGA AAC TGT GTC CTC TGC AAA CAG TGC GGA   265

 P   G   M   E   L   S   K   E   C   G   F   G   Y   G   E   D   A   Q   C   V    73
CCT GGC ATG GAG TTG TCC AAG GAA TGT GGC TTC GGC TAT GGG GAG GAT GCA CAG TGT GTG   325

 P   C   R   P   H   R   F   K   E   D   W   G   F   Q   K   C   K   P   C   A    93
CCC TGC AGG CCG CAC CGG TTC AAG GAA GAC TGG GGT TTC CAG AAG TGT AAG CCA TGT GCG   385

 D   C   A   L   V   N   R   F   Q   R   A   N   C   S   H   T   S   D   A   V   113
GAC TGT GCG CTG GTG AAC CGC TTT CAG AGG GCC AAC TGC TCA CAC ACC AGT GAT GCT GTC   445

 C   G   D   C   L   P   G   F   Y   R   K   T   K   L   V   G   F   Q   D   M   133
TGC GGG GAC TGC CTG CCA GGA TTT TAC CGG AAG ACC AAA CTG GTT GGT TTT CAA GAC ATG   505
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FIG.1B

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 E   C   V   P   C   G   D   P   P   P   P   Y   E   P   H   C   T   S   K   V   153
GAG TGT GTG CCC TGC GGA GAC CCA CCT CCT CCC TAC GAA CCA CAC TGT ACC AGC AAG GTG   565

 N   L   V   K   I   S   S   T   V   S   S   P   R   D   T   A   L   A   A   V   173
AAC CTT GTG AAG ATC TCC TCC ACC GTC TCC AGC CCT CGG GAC ACG GCG CTG GCT GCC GTC   625

 I   C   S   A   L   A   T   V   L   L   A   L   L   I   L   C   V   I   Y   C   193
ATC TGC AGT GCT CTG GCC ACG GTG CTG CTC GCC CTG CTC ATC CTG TGT GTC ATC TAC TGC   685

 K   R   Q   F   M   E   K   K   P   S   C   K   L   P   S   L   C   L   T   V   213
AAG AGG CAG TTC ATG GAG AAG AAA CCC AGC TGT AAG CTC CCA TCC CTC TGT CTC ACT GTG   745

 K   *                                                                           215
AAG TGA                                                                        751

GCTTGTTAGCATTGTCACCCAAGAGTTCTCAAGACACCTGGCTGAGACCTAAGACCTTTAGAGCATCAACAGCTACTTA  830

GAATACAAGATGCAGGAAAACGAGCCTCTTCAGGAATCTCAGGGCCTCCTAGGGATGCTGGCAAGGCTGTGATGTCTCA  909

AGGCTACCAGGAAAAAATAAAAGTTGTCTATACCCTAAAAAAAAAAAAAAAAAAAAAAAACATGCGGCCGC        981
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FIG.2

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GAATTCGGCACGAGGGCGTTTGGCGCGGAAGTGCTACCAAGCTGCGGAAAGCGTGAGTCTGGAGCACAGCACTGGCGAG   79

                                  M   A   L   K   V   L   P   L   H   R   T   V    12
TAGCAGGAATAAACACGTTTGGTGAGAGCC ATG GCA CTC AAG GTC CTA CCT CTA CAC AGG ACG GTG   145

 L   F   A   A   I   L   F   L   L   H   L   A   C   K   V   S   C   E   T   G    32
CTC TTC GCT GCC ATT CTC TTC CTA CTC CAC CTG GCA TGT AAA GTG AGT TGC GAA ACC GGA   205

 D   C   R   Q   Q   E   F   K   D   R   S   G   N   C   V   L   C   K   Q   C    52
GAT TGC AGG CAG CAG GAA TTC AAG GAT CGA TCT GGA AAC TGT GTC CTC TGC AAA CAG TGC   265

 G   P   G   M   E   L   S   K   E   C   G   F   G   Y   G   E   D   A   Q   C    72
GGA CCT GGC ATG GAG TTG TCC AAG GAA TGT GGC TTC GGC TAT GGG GAG GAT GCA CAG TGT   325

 V   P   C   R   P   H   R   F   K   E   D   W   G   F   Q   K   C   K   P   C    92
GTG CCC TGC AGG CCG CAC CGG TTC AAG GAA GAC TGG GGT TTC CAG AAG TGT AAG CCA TGT   385

 A   D   C   A   L   V   N   R   F   Q   R   A   N   C   S   H   T   S   D   A   112
GCG GAC TGT GCG CTG GTG AAC CGC TTT CAG AGG GCC AAC TGC TCA CAC ACC AGT GAT GCT   445

 V   C   G   D   C   L   P   G   F   Y   R   K   T   K   L   V   G   F   Q   D   132
GTC TGC GGG GAC TGC CTG CCA GGA TTT TAC CGG AAG ACC AAA CTG GTT GGT TTT CAA GAC   505

 M   E   C   V   P   C   G   D   P   P   P   P   Y   E   P   H   C   E   *       151
ATG GAG TGT GTG CCC TGC GGA GAC CCA CCT CCT CCC TAC GAA CCA CAC TGT GAG TGA       562

TGTGCCAAGTGGCAGCAGACCTTTAAAAAAAAAAGAAAAAAAAACAAACAAAAACAAAAAAAAAAAAAAAAAAAAA   641

ATTTCCGCGGCCGC                                                                 655
```

FIG.3A

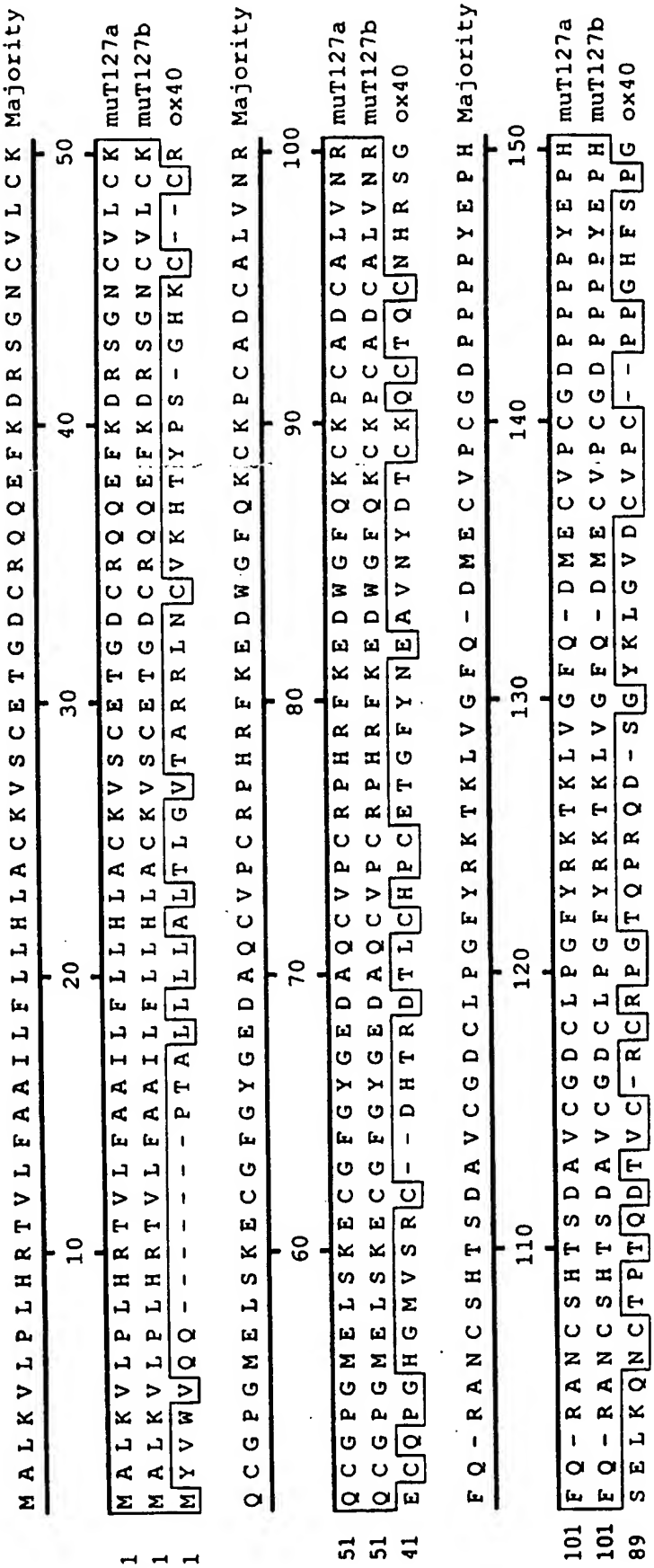

FIG.3B

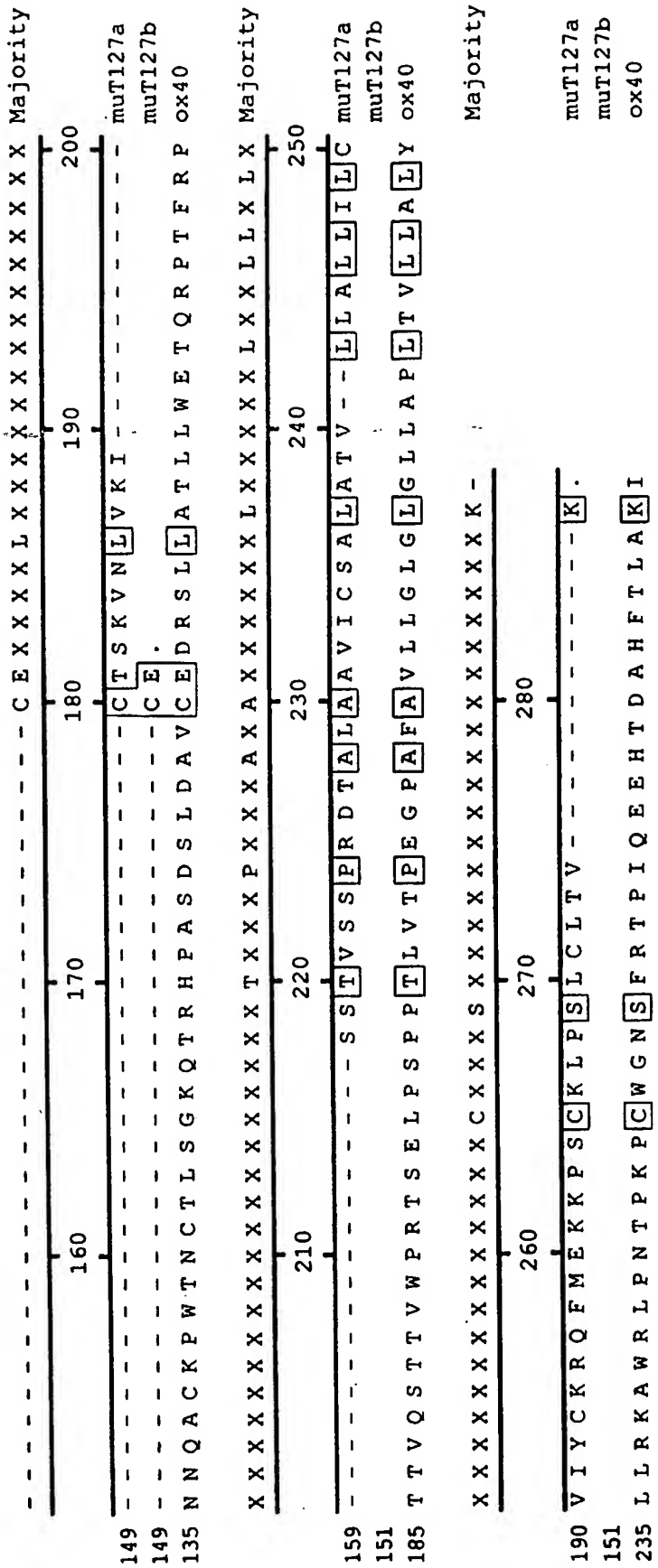
Majority
muT127a
muT127b
ox40
160
170
180
190
200
210
220
230
240
250
260
270
280

FIG.4A

```
FASTA searches a protein or DNA sequence data bank
version 2.0u53 July, 1996
Please cite:
W.R. Pearson & D.J. Lipman PNAS (1988) 85:2444-2448

inputs/nb589712.tmp :  215 aa
> T127Atm473200aa: 215 aa
vs  library
searching inputs/nb782215.tmp library

   423 residues in     1 sequences
The best scores are:                                   initn init1 opt
 Patent Protein W70387 - (untitled)                      982 982  982

>> Patent Protein W70387 - (untitled)                    (423 aa)
initn:  982  init1:  982  opt:  982
Smith-Waterman score: 982;    85.7% identity in 203 aa overlap

               10        20        30        40        50        60

T127A MALKVLPLHRTVLFAAILFLLHLACKVSCETGDCRQQEFKDRSGNCVLCKQCGPGMELSK

      X::::: .  ..:. ...: :.:::.:::::::::::::.::::::: :.:::::::::::

      MALKVLLEQEKTFFTLLVLLGYLSCKVTCETGDCRQQEFRDRSGNCVPCNQCGPGMELSK

               10        20        30        40        50        60
```

FIG.4B

```
                70        80        90        100       110       120
T127A ECGFGYGEDAQCVPCRPHRFKEDWGFQKCKPCADCALVNRFQRANCSHTSDAVCGDCLPG
      :::::::::::: :: :::::::::::::: :::.:::::.::: ::::.:::::::
      ECGFGYGEDAQCVTCRLHRFKEDWGFQKCKPCLDCAVVNRFQKANCSATSDAICGDCLPG
                70        80        90        100       110       120

               130       140       150       160       170       180
T127A FYRKTKLVGFQDMECVPCGDPPPPYEPHCTSKVNLVKISSTVSSPRDTALAAVICSALAT
      ::::::::::::::::::::::::::::::.::::::::.::::::::::::::::::
      FYRKTKLVGFQDMECVPCGDPPPPYEPHCASKVNLVKIASTASSPRDTALAAVICSALAT
               130       140       150       160       170       180

               190       200       210
T127A VLLALLILCVIYCKRQFMEKKPSCKLPSLCLTVKN
      ::::::::::::::::::::::X
      VLLALLILCVIYCKRQFMEKKPSWSLRSQDIQYNGSELSCLDPRQLHEYAHRACCQCRRD
               190       200       210       220       230       240
```

FIG.5A

```
 FASTA searches a protein or DNA sequence data bank
 version 2.0u53 July, 1996
Please cite:
 W.R. Pearson & D.J. Lipman PNAS (1988) 85:2444-2448

 inputs/nb504897.tmp :  981 aa
 > Atm472300: 981 aa
 vs  library
 searching inputs/nb658900.tmp library

   1496 residues in     1 sequences
The best scores are:                                   initn init1 opt
 Patent Nucleotide V33362 - (untitled)                  2575 2575 2861

>> Patent Nucleotide V33362 - (untitled)                    (1496 aa)
initn: 2575  init1: 2575  opt: 2861
Smith-Waterman score: 2888;    70.6% identity in 922 aa overlap


            40         50         60         70         80         90

Atm47 CCCCAGCCTCAAACTGCAGTCCGGCGCCGCGGGGCAGGACAAGGGGAAGGAATAAACACG

                                      X:..:. .. :      :. :::::: ::.

                                     GGGAACGTAGAACTCTCCAACAATAAATACA

                                           10         20         30
```

FIG.5B

```
          100        110        120        130        140        150
Atm47 TTTGGTGAGAGCCATGGCACTCAAGGTCCTACCTCTACACAGGACGGTGCTCTTCGCTGC
      :::::.:.:::.  ::::::. : ::.:: :::: . .::: ..:: .. : : :::.::
      TTTGATAAGAAAGATGGCTTTAAAAGTGCTACTAGAACAAGAGAAAACGTTTTTCACTCT
            40         50         60         70         80         90

          160        170        180        190        200        210
Atm47 CATTCTCTTCCTACTCCACCTGGCATGTAAAGTGAGTTGCGAAACCGGAGATTGCAGGCA
       .:. : :: :::   : :  :: :::::::::::: ::: ::::: ::::: :: ::.::
      TTTAGTATTACTAGGCTATTTGTCATGTAAAGTGACTTGTGAAACAGGAGACTGTAGACA
            100        110        120        130        140        150
```

FIG.5C

```
          220        230        240        250        260        270
Atm47 GCAGGAATTCAAGGATCGATCTGGAAACTGTGTCCTCTGCAAACAGTGCGGACCTGGCAT
      :::.:::::::.:::::.:::::::::::::::: : :::::: ::::: ::.::.::::: 
      GCAAGAATTCAGGGATCGGTCTGGAAACTGTGTTCCCTGCAACCAGTGTGGGCCAGGCAT
          160        170        180        190        200        210

          280        290        300        310        320        330
Atm47 GGAGTTGTCCAAGGAATGTGGCTTCGGCTATGGGGAGGATGCACAGTGTGTGCCCTGCAG
      ::::::::: :::::::::::::::::::::::::::::::::::::::: : ::: :
      GGAGTTGTCTAAGGAATGTGGCTTCGGCTATGGGGAGGATGCACAGTGTGTGACGTGCCG
          220        230        240        250        260        270
```

FIG.5D

```
          340        350        360        370        380        390
Atm47 GCCGCACCGGTTCAAGGAAGACTGGGGTTTCCAGAAGTGTAAGCCATGTGCGGACTGTGC
      :: :::: ::::::::::.:::::::: :::::::.:: ::::: ::: :::::: ::
      GCTGCACAGGTTCAAGGAGGACTGGGGCTTCCAGAAATGCAAGCCCTGTCTGGACTGCGC
          280        290        300        310        320        330

          400        410        420        430        440        450
Atm47 GCTGGTGAACCGCTTTCAGAGGGCCAACTGCTCACACACCAGTGATGCTGTCTGCGGGGA
      . ::::::::::::::::::.::: :: :: ::: :::::::::::: .::::::::::
      AGTGGTGAACCGCTTTCAGAAGGCAAATTGTTCAGCCACCAGTGATGCCATCTGCGGGGA
          340        350        360        370        380        390
```

FIG.5E

```
          460        470        480        490        500        510
Atm47 CTGCCTGCCAGGATTTTACCGGAAGACCAAACTGGTTGGTTTTCAAGACATGGAGTGTGT
      :::: ::::::::::::  ::::::: ::::: :: :: ::::::::::::::::::::
      CTGCTTGCCAGGATTTTATAGGAAGACGAAACTTGTCGGCTTTCAAGACATGGAGTGTGT
           400        410        420        430        440        450

          520        530        540        550        560        570
Atm47 GCCCTGCGGAGACCCACCTCCTCCCTACGAACCACACTGTACCAGCAAGGTGAACCTTGT
      ::: :: :::::::::.::::::: :::::::::.::::::.:::::::::: ::::: ::
      GCCTTGTGGAGACCCTCCTCCTCCTTACGAACCGCACTGTGCCAGCAAGGTCAACCTCGT
           460        470        480        490        500        510
```

FIG.5F

```
          580       590       600       610       620       630
Atm47 GAAGATCTCCTCCACCGTCTCCAGCCCTCGGGACACGGCGCTGGCTGCCGTCATCTGCAG
      ::::::: : ::::: : :::::::::.:::::::::::::::::::: ::::::::
      GAAGATCGCGTCCACGGCCTCCAGCCCACGGGACACGGCGCTGGCTGCCGTTATCTGCAG
          520       530       540       550       560       570
          640       650       660       670       680       690
Atm47 TGCTCTGGCCACGGTGCTGCTCGCCCTGCTCATCCTGTGTGTCATCTACTGCAAGAGGCA
      :::::::::::: :: ::::: ::::::::::::::::: :::::::::::: :: :::::.::
      CGCTCTGGCCACCGTCCTGCTGGCCCTGCTCATCCTCTGTGTCATCTATTGTAAGAGACA
          580       590       600       610       620       630
```

FIG.5G

```
          700        710        720        730        740        750
Atm47 GTTCATGGAGAAGAAACCCAGCTGTAAGCTCCCATCCCTCTGTCTCACTGTGAAGTGAGC
      ::: :::::::::::::::::::: .  :: : .:: :.  :.:. .:.::. :. : ::
      GTTTATGGAGAAGAAACCCAGCTGGTCTCTGCGGTCACA-GGACATTCAGTACAACG-GC
           640        650        660        670        680

          760        770        780        790        800        810
Atm47 TTGTTAGCATT-GTCACCCAAGAGTTCTCAAGACACCT-GGCTGAGACCTAAGA-CCTTT
      :  : :::. : :: .:  .: ::. :^:v.  :.::. :. :..: ::. ::: :::
      TC-TGAGCTGTCGTGTCTTGACAGACCTCAG--CTCCACGAATATGCCCACAGAGCCT--
    690        700        710        720        730        740
```

FIG.5H

```
                820       830       840       850       860       870
Atm47 AGAGCATCAACAGCTACTTAGAATACAAGATGCAGGAAAACGAGCCTCTTCAGGAATCTC

      ::. : : .:: .:  .::   :. ..:::::::.  ..::.::: : : : .. :::

      ---GCTGCCAGTGCCGCCGTGA---CTCAGTGCAGACCTGCGGGCCGGTGC-GCTTGCTC

               750       760       770       780       790

               880       890       900       910       920       930
Atm47 AGGGCCTCCTAGGGATGCTGGCAAGGCTGTGATGTCTCAAGGCTACCAGGAAAAAATAAA

      : :::..: : :: ::. .:::: :: .: : ::: : ::.: .: . .:....

      C---CATCCATGTGCTG-TGAGGAGGCC-TGCAGCCCCAA--C--CCGGCGACTCTTGGT

            800       810       820       830       840
```

FIG.5I

```
                940       950       960       970       980
Atm47 AGTTGTCTATACCCTAAAAAAAAAAAAAAAAAAAAAAAAACATGC--GGCCGC
      .:: :   :. :   ::. :.   :.. .. :.. ::.::: .:: ::   .::::
      TGTGGGGTGCATTCTGCAGCCAGTCTTCAGGCAAGAAACGCAGGCCCAGCCGGGGAGATG
     850       860       870       880       890       900

      GTGCCGACTTTCTTCGGATCCCTCACGCAGTCCATCTGTGGCGAGTTTTCAGATGCCTGG
     910       920       930       940       950       960
```

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: MILLENNIUM BIOTHERAPEUTICS, INC. et al.
(B) STREET: 620 MEMORIAL DRIVE
(C) CITY: CAMBRIDGE
(D) STATE: MASSACHUSETTS
(E) COUNTRY: US
(F) POSTAL CODE: 02139-4815
(G) TELEPHONE:
(H) TELEFAX:

(ii) TITLE OF INVENTION: NOVEL MOLECULES OF THE TNF RECEPTOR SUPERFAMILY AND USES THEREFOR _

(iii) NUMBER OF SEQUENCES: 17

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: LAHIVE & COCKFIELD, LLP
(B) STREET: 28 STATE STREET
(C) CITY: BOSTON
(D) STATE: MASSACHUSETTS
(E) COUNTRY: US
(F) ZIP: 02109

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: PCT/US99/
(B) FILING DATE: 27 JANUARY 1999
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 09/014,195
(B) FILING DATE: 27 JANUARY 1998

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: MANDRAGOURAS, AMY E.
(B) REGISTRATION NUMBER: 36,207
(C) REFERENCE/DOCKET NUMBER: MEI-009CPPC

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (617)227-7400
(B) TELEFAX: (617)742-4214

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 981 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 107..748

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
GAATTCGGCA CGAGGGCCGG CACCCCCGCG CCACCCCAGC CTCAAACTGC AGTCCGGCGC     60

CGCGGGGCAG GACAAGGGGA AGGAATAAAC ACGTTTGGTG AGAGCC ATG GCA CTC       115
                                                    Met Ala Leu
                                                      1

AAG GTC CTA CCT CTA CAC AGG ACG GTG CTC TTC GCT GCC ATT CTC TTC       163
Lys Val Leu Pro Leu His Arg Thr Val Leu Phe Ala Ala Ile Leu Phe
  5                  10                  15

CTA CTC CAC CTG GCA TGT AAA GTG AGT TGC GAA ACC GGA GAT TGC AGG       211
Leu Leu His Leu Ala Cys Lys Val Ser Cys Glu Thr Gly Asp Cys Arg
 20                  25                  30                  35

CAG CAG GAA TTC AAG GAT CGA TCT GGA AAC TGT GTC CTC TGC AAA CAG         2
Gln Gln Glu Phe Lys Asp Arg Ser Gly Asn Cys Val Leu Cys Lys Gln
                 40                  45                  50

TGC GGA CCT GGC ATG GAG TTG TCC AAG GAA TGT GGC TTC GGC TAT GGG       307
Cys Gly Pro Gly Met Glu Leu Ser Lys Glu Cys Gly Phe Gly Tyr Gly
             55                  60                  65

GAG GAT GCA CAG TGT GTG CCC TGC AGG CCG CAC CGG TTC AAG GAA GAC       355
Glu Asp Ala Gln Cys Val Pro Cys Arg Pro His Arg Phe Lys Glu Asp
         70                  75                  80

TGG GGT TTC CAG AAG TGT AAG CCA TGT GCG GAC TGT GCG CTG GTG AAC       403
Trp Gly Phe Gln Lys Cys Lys Pro Cys Ala Asp Cys Ala Leu Val Asn
 85                  90                  95

CGC TTT CAG AGG GCC AAC TGC TCA CAC ACC AGT GAT GCT GTC TGC GGG       451
Arg Phe Gln Arg Ala Asn Cys Ser His Thr Ser Asp Ala Val Cys Gly
100                 105                 110                 115

GAC TGC CTG CCA GGA TTT TAC CGG AAG ACC AAA CTG GTT GGT TTT CAA       499
Asp Cys Leu Pro Gly Phe Tyr Arg Lys Thr Lys Leu Val Gly Phe Gln
                120                 125                 130
```

```
GAC ATG GAG TGT GTG CCC TGC GGA GAC CCA CCT CCT CCC TAC GAA CCA      547
Asp Met Glu Cys Val Pro Cys Gly Asp Pro Pro Pro Tyr Glu Pro
            135                 140                 145

CAC TGT ACC AGC AAG GTG AAC CTT GTG AAG ATC TCC TCC ACC GTC TCC      595
His Cys Thr Ser Lys Val Asn Leu Val Lys Ile Ser Ser Thr Val Ser
        150                 155                 160

AGC CCT CGG GAC ACG GCG CTG GCT GCC GTC ATC TGC AGT GCT CTG GCC      643
Ser Pro Arg Asp Thr Ala Leu Ala Ala Val Ile Cys Ser Ala Leu Ala
    165                 170                 175

ACG GTG CTG CTC GCC CTG CTC ATC CTG TGT GTC ATC TAC TGC AAG AGG
691
Thr Val Leu Leu Ala Leu Leu Ile Leu Cys Val Ile Tyr Cys Lys Arg
180                 185                 190                 195

CAG TTC ATG GAG AAG AAA CCC AGC TGT AAG CTC CCA TCC CTC TGT CTC      739
Gln Phe Met Glu Lys Lys Pro Ser Cys Lys Leu Pro Ser Leu Cys Leu
                200                 205                 210

ACT GTG AAG TGAGCTTGTT AGCATTGTCA CCCAAGAGTT CTCAAGACAC              788
Thr Val Lys

CTGGCTGAGA CCTAAGACCT TTAGAGCATC AACAGCTACT TAGAATACAA GATGCAGGAA   848

AACGAGCCTC TTCAGGAATC TCAGGGCCTC CTAGGGATGC TGGCAAGGCT GTGATGTCTC   908

AAGGCTACCA GGAAAAAATA AAAGTTGTCT ATACCCTAAA AAAAAAAAAA AAAAAAAAAA   968

AACATGCGGC CGC                                                      981
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 214 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Ala Leu Lys Val Leu Pro Leu His Arg Thr Val Leu Phe Ala Ala
  1               5                  10                  15

Ile Leu Phe Leu Leu His Leu Ala Cys Lys Val Ser Cys Glu Thr Gly
            20                  25                  30

Asp Cys Arg Gln Gln Glu Phe Lys Asp Arg Ser Gly Asn Cys Val Leu
        35                  40                  45
```

```
Cys Lys Gln Cys Gly Pro Gly Met Glu Leu Ser Lys Glu Cys Gly Phe
     50                  55                  60

Gly Tyr Gly Glu Asp Ala Gln Cys Val Pro Cys Arg Pro His Arg Phe
 65                  70                  75                  80

Lys Glu Asp Trp Gly Phe Gln Lys Cys Lys Pro Cys Ala Asp Cys Ala
                 85                  90                  95

Leu Val Asn Arg Phe Gln Arg Ala Asn Cys Ser His Thr Ser Asp Ala
            100                 105                 110

Val Cys Gly Asp Cys Leu Pro Gly Phe Tyr Arg Lys Thr Lys Leu Val
        115                 120                 125

Gly Phe Gln Asp Met Glu Cys Val Pro Cys Gly Asp Pro Pro Pro Pro
    130                 135                 140

Tyr Glu Pro His Cys Thr Ser Lys Val Asn Leu Val Lys Ile Ser Ser
145                 150                 155                 160

Thr Val Ser Ser Pro Arg Asp Thr Ala Leu Ala Ala Val Ile Cys Ser
                165                 170                 175

Ala Leu Ala Thr Val Leu Leu Ala Leu Leu Ile Leu Cys Val Ile Tyr
            180                 185                 190

Cys Lys Arg Gln Phe Met Glu Lys Lys Pro Ser Cys Lys Leu Pro Ser
        195                 200                 205

Leu Cys Leu Thr Val Lys
    210
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 642 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..642

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
ATG GCA CTC AAG GTC CTA CCT CTA CAC AGG ACG GTG CTC TTC GCT GCC
48
```

```
Met Ala Leu Lys Val Leu Pro Leu His Arg Thr Val Leu Phe Ala Ala
 1               5                  10                  15

ATT CTC TTC CTA CTC CAC CTG GCA TGT AAA GTG AGT TGC GAA ACC GGA      96
Ile Leu Phe Leu Leu His Leu Ala Cys Lys Val Ser Cys Glu Thr Gly
            20                  25                  30

GAT TGC AGG CAG CAG GAA TTC AAG GAT CGA TCT GGA AAC TGT GTC CTC     144
Asp Cys Arg Gln Gln Glu Phe Lys Asp Arg Ser Gly Asn Cys Val Leu
        35                  40                  45

TGC AAA CAG TGC GGA CCT GGC ATG GAG TTG TCC AAG GAA TGT GGC TTC     192
Cys Lys Gln Cys Gly Pro Gly Met Glu Leu Ser Lys Glu Cys Gly Phe
    50                  55                  60

GGC TAT GGG GAG GAT GCA CAG TGT GTG CCC TGC AGG CCG CAC CGG TTC     240
Gly Tyr Gly Glu Asp Ala Gln Cys Val Pro Cys Arg Pro His Arg Phe
65                  70                  75                  80

AAG GAA GAC TGG GGT TTC CAG AAG TGT AAG CCA TGT GCG GAC TGT GCG     288
Lys Glu Asp Trp Gly Phe Gln Lys Cys Lys Pro Cys Ala Asp Cys Ala
                85                  90                  95

CTG GTG AAC CGC TTT CAG AGG GCC AAC TGC TCA CAC ACC AGT GAT GCT     336
Leu Val Asn Arg Phe Gln Arg Ala Asn Cys Ser His Thr Ser Asp Ala
            100                 105                 110

GTC TGC GGG GAC TGC CTG CCA GGA TTT TAC CGG AAG ACC AAA CTG GTT     384
Val Cys Gly Asp Cys Leu Pro Gly Phe Tyr Arg Lys Thr Lys Leu Val
        115                 120                 125

GGT TTT CAA GAC ATG GAG TGT GTG CCC TGC GGA GAC CCA CCT CCT CCC     432
Gly Phe Gln Asp Met Glu Cys Val Pro Cys Gly Asp Pro Pro Pro Pro
    130                 135                 140

TAC GAA CCA CAC TGT ACC AGC AAG GTG AAC CTT GTG AAG ATC TCC TCC     480
Tyr Glu Pro His Cys Thr Ser Lys Val Asn Leu Val Lys Ile Ser Ser
145                 150                 155                 160

ACC GTC TCC AGC CCT CGG GAC ACG GCG CTG GCT GCC GTC ATC TGC AGT     528
Thr Val Ser Ser Pro Arg Asp Thr Ala Leu Ala Ala Val Ile Cys Ser
                165                 170                 175

GCT CTG GCC ACG GTG CTG CTC GCC CTG CTC ATC CTG TGT GTC ATC TAC     576
Ala Leu Ala Thr Val Leu Leu Ala Leu Leu Ile Leu Cys Val Ile Tyr
            180                 185                 190

TGC AAG AGG CAG TTC ATG GAG AAG AAA CCC AGC TGT AAG CTC CCA TCC     624
Cys Lys Arg Gln Phe Met Glu Lys Lys Pro Ser Cys Lys Leu Pro Ser
        195                 200                 205

CTC TGT CTC ACT GTG AAG                                            642
Leu Cys Leu Thr Val Lys
    210
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 555 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..555

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
GAA ACC GGA GAT TGC AGG CAG CAG GAA TTC AAG GAT CGA TCT GGA AAC      8
Glu Thr Gly Asp Cys Arg Gln Gln Glu Phe Lys Asp Arg Ser Gly Asn
 1               5                  10                  15

TGT GTC CTC TGC AAA CAG TGC GGA CCT GGC ATG GAG TTG TCC AAG GAA     96
Cys Val Leu Cys Lys Gln Cys Gly Pro Gly Met Glu Leu Ser Lys Glu
            20                  25                  30

TGT GGC TTC GGC TAT GGG GAG GAT GCA CAG TGT GTG CCC TGC AGG CCG    144
Cys Gly Phe Gly Tyr Gly Glu Asp Ala Gln Cys Val Pro Cys Arg Pro
        35                  40                  45

CAC CGG TTC AAG GAA GAC TGG GGT TTC CAG AAG TGT AAG CCA TGT GCG    192
His Arg Phe Lys Glu Asp Trp Gly Phe Gln Lys Cys Lys Pro Cys Ala
    50                  55                  60

GAC TGT GCG CTG GTG AAC CGC TTT CAG AGG GCC AAC TGC TCA CAC ACC    240
Asp Cys Ala Leu Val Asn Arg Phe Gln Arg Ala Asn Cys Ser His Thr
65                  70                  75                  80

AGT GAT GCT GTC TGC GGG CAC TGC CTG CCA GGA TTT TAC CGG AAG ACC    288
Ser Asp Ala Val Cys Gly Asp Cys Leu Pro Gly Phe Tyr Arg Lys Thr
                85                  90                  95

AAA CTG GTT GGT TTT CAA GAC ATG GAG TGT GTG CCC TGC GGA GAC CCA    336
Lys Leu Val Gly Phe Gln Asp Met Glu Cys Val Pro Cys Gly Asp Pro
            100                 105                 110

CCT CCT CCC TAC GAA CCA CAC TGT ACC AGC AAG GTG AAC CTT GTG AAG    384
Pro Pro Pro Tyr Glu Pro His Cys Thr Ser Lys Val Asn Leu Val Lys
        115                 120                 125

ATC TCC TCC ACC GTC TCC AGC CCT CGG GAC ACG GCG CTG GCT GCC GTC    432
Ile Ser Ser Thr Val Ser Ser Pro Arg Asp Thr Ala Leu Ala Ala Val
    130                 135                 140
```

```
ATC TGC AGT GCT CTG GCC ACG GTG CTG CTC GCC CTG CTC ATC CTG TGT      480
Ile Cys Ser Ala Leu Ala Thr Val Leu Leu Ala Leu Leu Ile Leu Cys
145                 150                 155                 160

GTC ATC TAC TGC AAG AGG CAG TTC ATG GAG AAG AAA CCC AGC TGT AAG      528
Val Ile Tyr Cys Lys Arg Gln Phe Met Glu Lys Lys Pro Ser Cys Lys
                165                 170                 175

CTC CCA TCC CTC TGT CTC ACT GTG AAG                                  555
Leu Pro Ser Leu Cys Leu Thr Val Lys
            180                 185
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 655 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 110..559

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
GAATTCGGCA CGAGGGCGTT TGGCGCGGAA GTGCTACCAA GCTGCGGAAA GCGTGAGTCT     60

GGAGCACAGC ACTGGCGAGT AGCAGGAATA AACACGTTTG GTGAGAGCC ATG GCA        115
                                                      Met Ala
                                                        1

CTC AAG GTC CTA CCT CTA CAC AGG ACG GTG CTC TTC GCT GCC ATT CTC      163
Leu Lys Val Leu Pro Leu His Arg Thr Val Leu Phe Ala Ala Ile Leu
          5                  10                  15

TTC CTA CTC CAC CTG GCA TGT AAA GTG AGT TGC GAA ACC GGA GAT TGC      211
Phe Leu Leu His Leu Ala Cys Lys Val Ser Cys Glu Thr Gly Asp Cys
     20                  25                  30

AGG CAG CAG GAA TTC AAG GAT CGA TCT GGA AAC TGT GTC CTC TGC AAA      259
Arg Gln Gln Glu Phe Lys Asp Arg Ser Gly Asn Cys Val Leu Cys Lys
 35                  40                  45                  50

CAG TGC GGA CCT GGC ATG GAG TTG TCC AAG GAA TGT GGC TTC GGC TAT      307
Gln Cys Gly Pro Gly Met Glu Leu Ser Lys Glu Cys Gly Phe Gly Tyr
                 55                  60                  65

GGG GAG GAT GCA CAG TGT GTG CCC TGC AGG CCG CAC CGG TTC AAG GAA      355
Gly Glu Asp Ala Gln Cys Val Pro Cys Arg Pro His Arg Phe Lys Glu
             70                  75                  80
```

```
GAC TGG GGT TTC CAG AAG TGT AAG CCA TGT GCG GAC TGT GCG CTG GTG      403
Asp Trp Gly Phe Gln Lys Cys Lys Pro Cys Ala Asp Cys Ala Leu Val
         85                  90                  95

AAC CGC TTT CAG AGG GCC AAC TGC TCA CAC ACC AGT GAT GCT GTC TGC      451
Asn Arg Phe Gln Arg Ala Asn Cys Ser His Thr Ser Asp Ala Val Cys
    100                 105                 110

GGG GAC TGC CTG CCA GGA TTT TAC CGG AAG ACC AAA CTG GTT GGT TTT      499
Gly Asp Cys Leu Pro Gly Phe Tyr Arg Lys Thr Lys Leu Val Gly Phe
115                 120                 125                 130

CAA GAC ATG GAG TGT GTG CCC TGC GGA GAC CCA CCT CCT CCC TAC GAA      547
Gln Asp Met Glu Cys Val Pro Cys Gly Asp Pro Pro Pro Pro Tyr Glu
                135                 140                 145

CCA CAC TGT GAG TGATGTGCCA AGTGGCAGCA GACCTTTAAA AAAAAAAGAA          599
Pro His Cys Glu
            150

AAAAAAACAA ACAAAAACAA AAAAAAAAAA AAAAAAAAAA AAATTTCCGC GGCCGC       655
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 150 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Ala Leu Lys Val Leu Pro Leu His Arg Thr Val Leu Phe Ala Ala
  1               5                  10                  15

Ile Leu Phe Leu Leu His Leu Ala Cys Lys Val Ser Cys Glu Thr Gly
            20                  25                  30

Asp Cys Arg Gln Gln Glu Phe Lys Asp Arg Ser Gly Asn Cys Val Leu
         35                  40                  45

Cys Lys Gln Cys Gly Pro Gly Met Glu Leu Ser Lys Glu Cys Gly Phe
     50                  55                  60

Gly Tyr Gly Glu Asp Ala Gln Cys Val Pro Cys Arg Pro His Arg Phe
 65                  70                  75                  80

Lys Glu Asp Trp Gly Phe Gln Lys Cys Lys Pro Cys Ala Asp Cys Ala
                85                  90                  95

Leu Val Asn Arg Phe Gln Arg Ala Asn Cys Ser His Thr Ser Asp Ala
            100                 105                 110
```

```
Val Cys Gly Asp Cys Leu Pro Gly Phe Tyr Arg Lys Thr Lys Leu Val
        115                 120                 125

Gly Phe Gln Asp Met Glu Cys Val Pro Cys Gly Asp Pro Pro Pro Pro
    130                 135                 140

Tyr Glu Pro His Cys Glu
145                 150
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 450 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..450

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
ATG GCA CTC AAG GTC CTA CCT CTA CAC AGG ACG GTG CTC TTC GCT GCC      48
Met Ala Leu Lys Val Leu Pro Leu His Arg Thr Val Leu Phe Ala Ala
  1               5                  10                  15

ATT CTC TTC CTA CTC CAC CTG GCA TGT AAA GTG AGT TGC GAA ACC GGA      96
Ile Leu Phe Leu Leu His Leu Ala Cys Lys Val Ser Cys Glu Thr Gly
             20                  25                  30

GAT TGC AGG CAG CAG GAA TTC AAG GAT CGA TCT GGA AAC TGT GTC CTC     144
Asp Cys Arg Gln Gln Glu Phe Lys Asp Arg Ser Gly Asn Cys Val Leu
         35                  40                  45

TGC AAA CAG TGC GGA CCT GGC ATG GAG TTG TCC AAG GAA TGT GGC TTC     192
Cys Lys Gln Cys Gly Pro Gly Met Glu Leu Ser Lys Glu Cys Gly Phe
     50                  55                  60

GGC TAT GGG GAG GAT GCA CAG TGT GTG CCC TGC AGG CCG CAC CGG TTC     240
Gly Tyr Gly Glu Asp Ala Gln Cys Val Pro Cys Arg Pro His Arg Phe
 65                  70                  75                  80

AAG GAA GAC TGG GGT TTC CAG AAG TGT AAG CCA TGT GCG GAC TGT GCG     288
Lys Glu Asp Trp Gly Phe Gln Lys Cys Lys Pro Cys Ala Asp Cys Ala
                 85                  90                  95
```

```
CTG GTG AAC CGC TTT CAG AGG GCC AAC TGC TCA CAC ACC AGT GAT GCT     336
Leu Val Asn Arg Phe Gln Arg Ala Asn Cys Ser His Thr Ser Asp Ala
          100                 105                 110

GTC TGC GGG GAC TGC CTG CCA GGA TTT TAC CGG AAG ACC AAA CTG GTT     384
Val Cys Gly Asp Cys Leu Pro Gly Phe Tyr Arg Lys Thr Lys Leu Val
        115                 120                 125

GGT TTT CAA GAC ATG GAG TGT GTG CCC TGC GGA GAC CCA CCT CCT CCC     432
Gly Phe Gln Asp Met Glu Cys Val Pro Cys Gly Asp Pro Pro Pro Pro
    130                 135                 140

TAC GAA CCA CAC TGT GAG                                            450
Tyr Glu Pro His Cys Glu
145                 150
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 363 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..363

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
GAA ACC GGA GAT TGC AGG CAG CAG GAA TTC AAG GAT CGA TCT GGA AAC      48
Glu Thr Gly Asp Cys Arg Gln Gln Glu Phe Lys Asp Arg Ser Gly Asn
  1               5                  10                  15

TGT GTC CTC TGC AAA CAG TGC GGA CCT GGC ATG GAG TTG TCC AAG GAA      96
Cys Val Leu Cys Lys Gln Cys Gly Pro Gly Met Glu Leu Ser Lys Glu
            20                  25                  30

TGT GGC TTC GGC TAT GGG GAG GAT GCA CAG TGT GTG CCC TGC AGG CCG     144
Cys Gly Phe Gly Tyr Gly Glu Asp Ala Gln Cys Val Pro Cys Arg Pro
        35                  40                  45

CAC CGG TTC AAG GAA GAC TGG GGT TTC CAG AAG TGT AAG CCA TGT GCG     192
His Arg Phe Lys Glu Asp Trp Gly Phe Gln Lys Cys Lys Pro Cys Ala
    50                  55                  60

GAC TGT GCG CTG GTG AAC CGC TTT CAG AGG GCC AAC TGC TCA CAC ACC     240
Asp Cys Ala Leu Val Asn Arg Phe Gln Arg Ala Asn Cys Ser His Thr
65                  70                  75                  80
```

```
AGT GAT GCT GTC TGC GGG GAC TGC CTG CCA GGA TTT TAC CGG AAG ACC      288
Ser Asp Ala Val Cys Gly Asp Cys Leu Pro Gly Phe Tyr Arg Lys Thr
                 85                  90                  95

AAA CTG GTT GGT TTT CAA GAC ATG GAG TGT GTG CCC TGC GGA GAC CCA      336
Lys Leu Val Gly Phe Gln Asp Met Glu Cys Val Pro Cys Gly Asp Pro
            100                 105                 110

CCT CCT CCC TAC GAA CCA CAC TGT GAG                                  363
Pro Pro Pro Tyr Glu Pro His Cys Glu
        115                 120
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
Met Ala Leu Lys Val Leu Pro Leu His Arg Thr Val Leu Phe Ala Ala
1               5                  10                  15

Ile Leu Phe Leu Leu His Leu Ala Cys Lys Val Ser Cys
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Ala Leu Ala Ala Val Ile Cys Ser Ala Leu Ala Thr Val Leu Leu Ala
1               5                  10                  15

Leu Leu Ile Leu Cys Val Ile Tyr Cys
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Cys Arg Gln Gln Glu Phe Lys Asp Arg Ser Gly Asn Cys Val Leu Cys
1 5 10 15

Lys Gln Cys Gly Pro Gly Met Glu Leu Ser Lys Glu Cys Gly Phe Gly
20 25 30

Tyr Gly Glu Asp Ala Gln Cys
35

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Cys Arg Pro His Arg Phe Lys Glu Asp Trp Gly Phe Gln Lys Cys Lys
1 5 10 15

Pro Cys Ala Asp Cys Ala Leu Val Asn Arg Phe Gln Arg Ala Asn Cys
20 25 30

Ser His Thr Ser Asp Ala Val Cys
35 40

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Met Ala Leu Lys Val Leu Pro Leu His Arg Thr Val Leu Phe Ala Ala
1 5 10 15

Ile Leu Phe Leu Leu His Leu Ala Cys Lys Val Ser Cys
20 25

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Cys Arg Gln Gln Glu Phe Lys Asp Arg Ser Gly Asn Cys Val Leu Cys
1 5 10 15

Lys Gln Cys Gly Pro Gly Met Glu Leu Ser Lys Glu Cys Gly Phe Gly
20 25 30

Tyr Gly Glu Asp Ala Gln Cys
35

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Cys Arg Pro His Arg Phe Lys Glu Asp Trp Gly Phe Gln Lys Cys Lys
1 5 10 15

Pro Cys Ala Asp Cys Ala Leu Val Asn Arg Phe Gln Arg Ala Asn Cys
20 25 30

Ser His Thr Ser Asp Ala Val Cys
35 40

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 2
(D) OTHER INFORMATION: /note= "Xaa is four or six amino acids."

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 6
(D) OTHER INFORMATION: /note= "Xaa is 5 or 10 amino acids."

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 8
(D) OTHER INFORMATION: /note= "Xaa is 0 or 2 amino acids."

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 10
(D) OTHER INFORMATION: /note= "Xaa is 7 or 11 amino acids."

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 12
(D) OTHER INFORMATION: /note= "Xaa is 4 or 6 amino acids."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Cys Xaa Phe Tyr His Xaa Cys Xaa Cys Xaa Cys Xaa Asp Asn Glu Gln
1 5 10 15

Ser Lys Pro Xaa Xaa Cys
20

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 2
(D) OTHER INFORMATION: /note= "Xaa is between four and fourteen amino acids."

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 4
(D) OTHER INFORMATION: /note= "Xaa is between 0 and 2 amino acids."

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 6
(D) OTHER INFORMATION: /note= "Xaa is between 2 and 4 amino acids."

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 8
(D) OTHER INFORMATION: /note= "Xaa is between 6 and 12 amino acids."

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 10
(D) OTHER INFORMATION: /note= "Xaa is between 6 and 10 amino acids."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Cys Xaa Cys Xaa Cys Xaa Cys Xaa Cys Xaa Cys
1 5 10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/01679

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 7.1, 29, 69.1, 320.1, 325; 514/1+, 12; 530/350+, 387.1, 388.22, 412; 536/ 23.4, 23.5; 800/13

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Chemical Abstracts, MPSRCH, EMBL, GENBANK

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,395,760 A (SMITH et al) 07 March 1995, col. 2, line 38 to col. 3, line.	1-69
Y	US 5,447,851 A (BEUTLER et al) 05 September 1995, col. 4, line 49 to col. 5, line 49.	1-69
A	US 5,656,272 A (LE et al) 12 August 1997.	1-69
A	US 5,698,195 A (LE et al) 16 December 1997.	1-69
Y	GRAY, P.W. et al. Cloning of Human Tumor Necrosis Factor (TNF) Receptor cDNA and expression of Recombinant Soluble TNF-Binding Protein. Proceeding of the National Academy of Sciences (USA). October 1990, Vol. 87, pages 7380-7384, see especially pages 7381-7384.	1-69

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)		
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
05 MAY 1999	26 MAY 1999

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Deborah Crouch JOYCE BRIDGERS PARALEGAL SPECIALIST CHEMICAL MATRIX
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Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/01679

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	AMMANN, P. et al. Transgenic Mice Expressing Soluble Tumor Necrosis Factor-Receptor are Protected Against Bone Loss Caused by Estrogen Deficiency. Journal of Clinical Investigation. 1997, Vol. 99, No. 7, pages 1699-1703, see especially pages 1701-1702.	24 and 25

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/01679

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6): C12Q 1/68, 1/02; C12P 21/06; C12N 5/00, 5/00; A61K 31/00, 38/00; C07K 1/00, 16/00; C07H 21/04; A01K 67/027

A. CLASSIFICATION OF SUBJECT MATTER:
US CL : 435/6, 7.1, 29, 69.1, 320.1, 325; 514/1+, 12; 530/350+, 387.1, 388.22, 412; 536/ 23.4, 23.5; 800/13